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TITLE: Could HER2 heterogeneity open new therapeutic options in patients with HER2- primary breast cancer?

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14. ABSTRACT The purpose of this study is to determine if targeted imaging with a HER2-targeting PET tracer can detect HER2-positive metastases in patients with HER2-negative primary breast cancer. Twenty-three patients have been accrued to the trial using ⁸⁹ Zr-trastuzumab as the imaging agent. Nine patients demonstrated suspicious foci on ⁸⁹ Zr-trastuzumab PET/CT. Three of nine patients with suspicious foci have now been found to have biopsy-proven HER2-positive metastases. Thus, ⁸⁹ Zr-trastuzumab PET/CT may detect HER2-positive metastases in patients with presumed HER2-negative primary breast cancer. This is an initial proof-of-concept that targeted imaging may help identify patients eligible for targeted therapies. However, six of nine patients have had biopsies demonstrating HER2-negative metastases, despite ⁸⁹ Zr-trastuzumab positivity. Two manuscripts have been published on the work with ⁸⁹ Zr-trastuzumab. These false-positive results limit the ability of ⁸⁹ Zr-trastuzumab to be translated into clinical use and a more specific radiotracer will be needed. A first-in-human trial of ⁸⁹ Zr-pertuzumab PET/CT was performed in six patients with HER2-positive metastatic breast cancer, demonstrating safety, dosimetry, and effectiveness of ⁸⁹ Zr-pertuzumab to image HER2-positive disease. One manuscripts has been submitted for publication on the work with ⁸⁹ Zr-pertuzumab. We will now return to evaluating patients with HER2-negative primary breast cancer, using ⁸⁹ Zr-pertuzumab as the imaging agent.					
15. SUBJECT TERMS Breast cancer, HER2, tumor heterogeneity, PET/CT, ⁸⁹ Zr-trastuzumab					
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1. INTRODUCTION:

Human epidermal growth factor receptor 2 (HER2) is a highly valuable biomarker in breast cancer, and its expression directly influences treatment. Growing evidence suggests that HER2 expression may change between the primary breast malignancy and metastases. This is an example of tumor heterogeneity. Inaccurate knowledge of receptor status in metastases due to tumor heterogeneity may lead to suboptimal treatment of metastatic breast cancer.

Our central hypothesis is that imaging with a targeted HER2 radiotracer will allow us to identify patients with HER2-negative primary breast cancers who develop HER2-positive metastases, and who may benefit from the addition of HER2 therapy.

2. KEYWORDS:

Breast cancer
Human epidermal growth factor receptor 2 (HER2)
Tumor heterogeneity
PET/CT
Targeted imaging
⁸⁹Zr-trastuzumab

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Task 1. Submission and approval of regulatory documents

- 1a. IND for ⁸⁹Zr-trastuzumab
- 1b. IRB Protocol

Task 2. Determine the proportion of patients with HER2- primary breast cancer who develop imagable HER2+ metastases using a targeted HER2 radiotracer

- 2a. Accrue 23 patients at a rate of 1-2 per month
- 2b. Confirm HER2- status of archived pathology tissue samples
- 2c. ⁸⁹Zr-trastuzumab PET/CT to identify patients suspicious for HER2+ metastases
- 2d. Biopsies to confirm HER2+ malignancy
- 2e. Interim statistical analyses as predefined accrual numbers are met

Task 3. Among patients with HER2+ metastases discovered in Specific Aim #1, determine if HER2-targeted therapy results in a measurable treatment response

- 3a. Pre-treatment baseline disease assessments with FDG PET and CT
- 3b. HER2-targeted systemic therapy
- 3c. Post-treatment disease assessments with FDG PET and CT
- 3d. Final statistical analyses

Could HER2 heterogeneity open new therapeutic options in patients with HER2- primary breast cancer?

Task 4. Submission and approval of regulatory documents for ^{89}Zr -pertuzumab. Six-to-ten patient first-in-human study of ^{89}Zr -pertuzumab in patients with HER2+ breast cancer.

- 1a. IND for ^{89}Zr -trastuzumab
- 1b. IRB Protocol
- 1c. Six-to-ten patient first-in-human study of ^{89}Zr -pertuzumab

Task 5. Determine the proportion of patients with HER2- primary breast cancer who develop imagable HER2+ metastases using ^{89}Zr - pertuzumab

- 2a. Accrue 21 patients at a rate of 1-2 patients per month
- 2b. Confirm HER2- status of archived pathology tissue samples
- 2c. ^{89}Zr -pertuzumab PET/CT to identify patients suspicious for HER2+ metastases
- 2d. Biopsies to confirm HER2+ malignancy

Task 6. Among patients with HER2+ metastases discovered in Task 5, determine if HER2 targeted therapy results in a measurable treatment response

- 3a. Pre-treatment baseline disease assessments with FDG PET and CT
- 3b. HER2 targeted systemic therapy
- 3c. Post-treatment disease assessments with FDG PET and CT

What was accomplished under these goals?

Task 1. Submission and approval of regulatory documents

- 1a. IND for ^{89}Zr -trastuzumab
IND for ^{89}Zr was completed and maintained
- 1b. IRB Protocol
IRB for study protocol was completed and maintained.

Task 2. Determine the proportion of patients with HER2- primary breast cancer who develop imagable HER2+ metastases using a targeted HER2 radiotracer

- 2a. Accrue 23 patients at a rate of 1-2 per month
23 patients were accrued to this portion of the protocol as of 30-Sep-2016.
- 2b. Confirm HER2- status of archived pathology tissue samples
HER2- status of archived pathology samples was confirmed for all patients.
- 2c. ^{89}Zr -trastuzumab PET/CT to identify patients suspicious for HER2+ metastases
 ^{89}Zr -trastuzumab PET/CT was performed in all patients. Nine patients had ^{89}Zr -trastuzumab foci suspicious for HER2+ disease.
- 2d. Biopsies to confirm HER2+ malignancy

Could HER2 heterogeneity open new therapeutic options in patients with HER2- primary breast cancer?

Biopsies were performed in all nine patients with ^{89}Zr -trastuzumab foci suspicious for HER2+ disease.

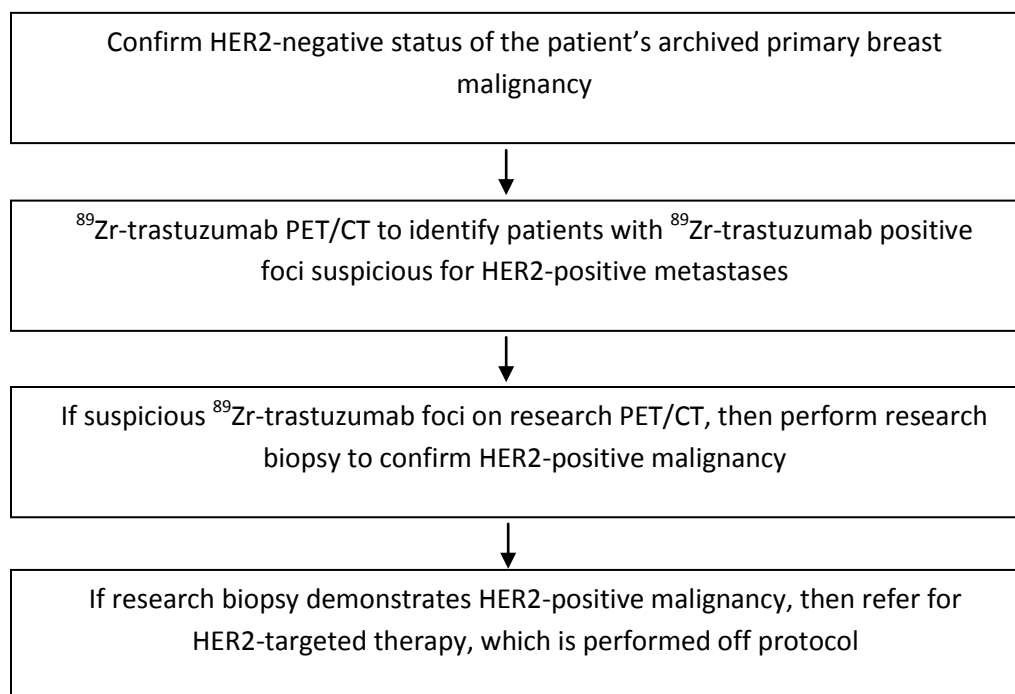
- Three of nine were positive for HER2+ disease on pathology (true positives)
- Six of nine were not positive for HER2+ disease on pathology at any site (false positives)

2e. Interim statistical analyses as predefined accrual numbers are met
Interim statistical analyses were performed.

Task 3. Among patients with HER2+ metastases discovered in Specific Aim #1, determine if HER2-targeted therapy results in a measurable treatment response

- 3a. Pre-treatment baseline disease assessments with FDG PET and CT
Pre-treatment baseline disease assessments were performed in the two patients with biopsy-proven, HER2+ metastases.
- 3b. HER2-targeted systemic therapy
HER2-targeted systemic therapy was performed in the two patients with biopsy-proven, HER2+ metastases.
- 3c. Post-treatment disease assessments with FDG PET and CT
Post-treatment disease assessments were performed in the two patients with biopsy-proven, HER2+ metastases. Both patients demonstrated response to HER2-targeted therapy (one complete, one partial).

Tasks 1- 3 has resulted in 20 patients completing the protocol with ^{89}Zr -trastuzumab PET/CT. All twenty had pathologic retesting that confirmed HER2-negative primary breast cancer. Nine demonstrated suspicious foci on ^{89}Zr -trastuzumab PET/CT. Of these nine with suspicious foci, three had biopsy proven HER2-positive metastases and went on to benefit from HER2 targeted therapy. Six of the nine patients with suspicious foci had biopsy without evidence of HER2-positive disease, and were considered false positive ^{89}Zr -trastuzumab PET foci. A protocol schema for these 20 patients is described in Figure 1. A tabulated summary of the results from these 20 patients is described in Table 1.

Figure 1: Protocol Schema**Table 1: Patient Demographics and Study Results**

Patient	Age	Sites of distant metastases at enrollment	Confirmatory IHC of primary breast cancer	Suspicious foci on ⁸⁹ Zr-trastuzumab PET/CT?	Image guided biopsy results
1	46	Bone, Liver	HER2 0	None	
2	41	Nodes	HER2 1+	Nodes (SUVmax 4.6)	HER2 IHC 3+
3	58	Bone, Liver, Nodes	HER2 0	Bone (SUV max7.1)	HER2 IHC 1+
4	69	Bone, Liver, Nodes	HER2 0	None	
5	38	Bone, Liver, Nodes	HER2 1+	Bone (SUVmax 5.9)	HER2 IHC 2+ FISH Failed MSK-IMPACT

Could HER2 heterogeneity open new therapeutic options in patients with HER2- primary breast cancer?

					amplified
6	42	Nodes, Adrenal	HER2 1+	Adrenal (SUVmax 9.2)	HER2 IHC 2+ FISH 1.4
7	83	Bone, Liver, Nodes, Lung	HER2 1+	Bone (SUVmax 9.7)	HER2 IHC 1+
8	54	Bone, Liver, Nodes, Lung	HER2 0	None	
9	48	Bone, Liver, Nodes, Pleura	HER2 1+	None	
10	54	Bone, Nodes	HER2 1+	None	
11	40	Bone, Liver, Nodes, Chest Wall	HER2 0	Bone (SUV 10.1) and Nodal (SUV 5.1)	HER2 IHC 2+ FISH 1.6
12	56	Liver	HER2 1+	None	
13	43	Nodes, Lung	HER2 1+	None	
14	47	Liver, Nodes, Pleura	HER2 2+ FISH 1.4	Liver (SUV 10.9) and Pleura (SUV 6.9)	HER2 IHC 2+ FISH 2.4
15	50	Bone, Liver	HER2 1+	None	
16	49	Bone, Liver	HER2 0	None	
17	51	Bone, Nodes, Pleura	HER2 0	Lung (SUV 11.3)	HER2 1+
18	48	Bone, Liver, Nodes	HER2 1+	None	
19	60	Liver	HER2 1+	None	
20	58	Bone, Liver, Nodes	HER2 1+	Liver (SUV 15.7)	HER2 1+

The major result from these 20 patients was the demonstration of unsuspected HER2-positive metastases in patients with HER2-negative primary breast malignancy. This resulted in addition of HER2-targeted therapy for these patients, with successful therapy response.

One example of successful identification of unsuspected HER2-positive metastases was patient 2 (Figure 2). Patient 2 was a 41-year-old woman who had undergone a right mastectomy in March 2014 with pathology demonstrating an ER-positive / HER2-negative primary invasive ductal carcinoma (Fig. 2A). Thus, she was treated initially with ovarian suppression and tamoxifen, and then with fulvestrant and aromatase inhibition, but with mixed treatment responses and residual disease as determined by CT. In November 2014 CT imaging demonstrated enlarged thoracic nodes consistent with malignancy. ^{89}Zr -trastuzumab PET/CT was performed in December 2014, and demonstrated ^{89}Zr -trastuzumab -avid thoracic nodes (Fig. 2B). The most avid node was a right supraclavicular node (SUVmax 4.6). In this patient biopsy of a right supraclavicular node demonstrated HER2 IHC of 3+ (Fig. 2C). This was considered a true positive ^{89}Zr -trastuzumab focus for a HER2-positive distant metastasis. She was then switched to treatment with trastuzumab, pertuzumab, and paclitaxel. Follow-up imaging for evaluation of treatment response was performed with CT of the chest, abdomen, and pelvis, which demonstrated resolution of previously enlarged lymph nodes as well as the absence of new lesions consistent with a complete response (Fig. 2D).

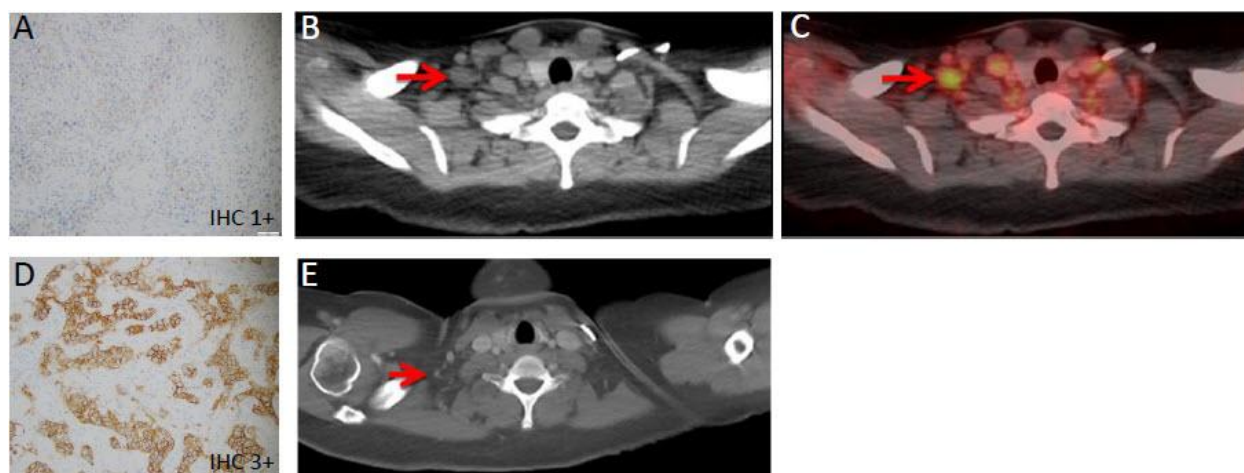


Figure 2: 41-year-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma and recurrence in thoracic nodes. (A) HER2 IHC (at 20X magnification) of the primary breast malignancy was 1+, consistent with HER2-negative malignancy. (B) Axial CT and fused ^{89}Zr -trastuzumab PET/CT demonstrated ^{89}Zr -trastuzumab avidity in enlarged right supraclavicular nodes (arrows, SUVmax 4.6) and left internal mammary nodes (not shown). (C) Biopsy of a right supraclavicular node demonstrated metastatic breast carcinoma with HER2 3+ on IHC (at 20X magnification), consistent with HER2-positive disease as defined by ASCO guidelines. The patient began systemic treatment including trastuzumab and pertuzumab. (D) Follow-up axial CT after two months of treatment demonstrated resolution of the nodes on CT (short arrow).

A second example of successful identification of unsuspected HER2-positive metastases was patient 5 (Figure 3). Patient 5 was a 38-year-old woman who had undergone a left mastectomy in July 2011 with pathology demonstrating an ER-positive / HER2-negative primary invasive ductal carcinoma (Fig. 3A). Osseous, hepatic, and nodal metastases were identified beginning in October 2013, and were progressing despite systemic therapy, including fulvestrant, leuprolide, and a novel clinical trial therapeutic. In April 2015, ^{89}Zr -trastuzumab PET/CT was performed, which demonstrated multiple suspicious osseous foci (Figs. 3B). The most avid foci were in the right ilium and right proximal femur (SUVmax 5.9). In this patient, biopsy of the right ilium demonstrated equivocal HER2 findings based on ASCO guidelines. HER2 IHC was graded as equivocal (Fig. 3C), due to a small percentage of cells with incomplete membranous staining. HER2 FISH failed after repeated attempts. Given the equivocal results, further testing was performed with the MSK-IMPACT assay. *ERBB2* fold change on MSK-IMPACT was 2.6 (≥ 2.0 is considered positive for amplification; Fig. 3D). Therefore, this was considered a true positive ^{89}Zr -trastuzumab focus for a HER2-positive distant metastasis (14). Patient 5 was then switched to treatment with trastuzumab, pertuzumab and docetaxel. Follow-up imaging for evaluation of treatment response was performed by FDG PET/CT. Following therapy, there was a decrease in size and FDG-avidity of liver and nodal metastases, as well as decreased FDG-avidity of osseous lesions, representing a partial response to treatment (Figs. 4A and 4B). In the liver, multiple FDG-avid lesions resolved after treatment, while a residual lesion in segment 4 decreased from SUVmax 8.2 to 5.6. In the osseous system, multiple FDG-avid lesions resolved after treatment, while other residual lesions decreased, such as the T11 vertebral body which decreased from SUVmax 9.1 to 4.7.

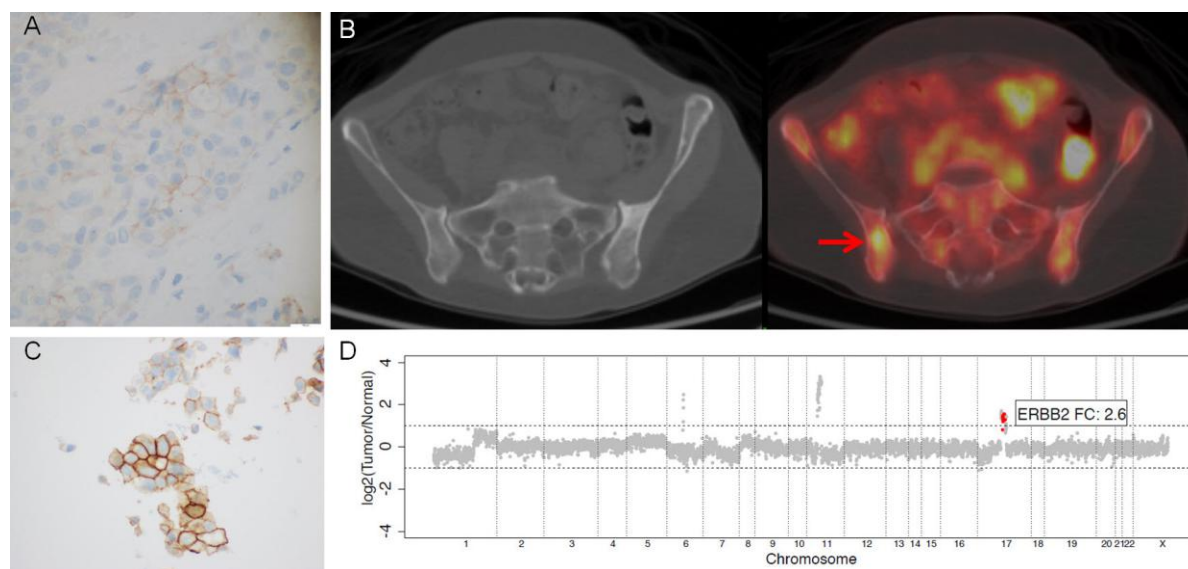


Figure 3: 38-year-old woman with primary ER-positive / HER2-negative invasive ductal breast carcinoma. (A) HER2 IHC (at 400X magnification) of the primary breast malignancy was 1+, consistent with HER2-negative malignancy. (B) Axial CT and fused ^{89}Zr -trastuzumab PET/CT demonstrated ^{89}Zr -trastuzumab avidity in the right ilium (arrow, SUVmax 5.9). Avidity in the bowel is considered

physiologic. (C) Biopsy of the right ilium demonstrated metastatic breast carcinoma with HER2 2+ on IHC (at 400X magnification), considered equivocal by ASCO guidelines (11). (D) MSK-IMPACT copy number plot demonstrating HER2 amplification. Each dot represents a probeset and the values on the y-axis show the log₂ transformed ratio of tumor vs normal. *ERBB2* fold change was 2.6, consistent with HER2-positive disease.

The major limitation from Tasks 1-3 was that six patients demonstrated ⁸⁹Zr-trastuzumab foci on PET/CT which were expected to be HER2-positive disease, yet biopsy and pathologic analysis still yielded HER2-negative results. These results were either due to false positive ⁸⁹Zr-trastuzumab PET/CT scans, or false negative biopsy results. Given it was likely that false negative biopsies could result in this many discordant findings, we are presuming that their were false positive ⁸⁹Zr-trastuzumab PET/CT scans.

An example of a presumed false positive ⁸⁹Zr-trastuzumab PET/CT scans was patient 7 (Figure 4). Patient 7 was an 83 year-old woman who presented in June 2010 with metastatic ER-positive, HER2-negative invasive ductal breast cancer (Fig. 4A). Despite several courses of chemotherapy, she had persistent osseous, hepatic, nodal, and lung metastases on FDG PET/CT in 2015. ⁸⁹Zr-trastuzumab PET/CT was performed in July 2015, and demonstrated multiple suspicious osseous foci (Figs. 4B and 4C). The most avid osseous lesion was in the cervical spine (SUVmax 9.7), so a lesion more easily assessable in the proximal left femur (SUVmax 7.7) was chosen for biopsy. Pathology demonstrated metastatic breast cancer but HER2 IHC was only 1+, consistent with HER2-negative disease (Fig. 4D). This was considered a false positive ⁸⁹Zr-trastuzumab focus.

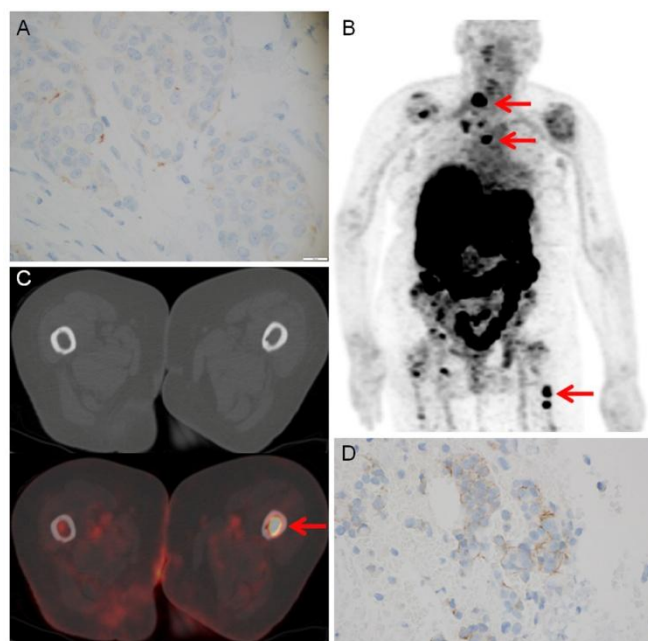


Figure 4. 83-year-old woman with primary ER-positive / HER2-negative invasive ductal breast carcinoma. (A) HER IHC (at 400X magnification) of the primary breast malignancy was 1+, consistent with HER2-negative malignancy. (B) ⁸⁹Zr-trastuzumab MIP demonstrates several foci of ⁸⁹Zr-trastuzumab avidity that localize to osseous structures. Avidity in the liver and bowel is considered physiologic. (C) Axial CT and fused ⁸⁹Zr-trastuzumab PET/CT demonstrated ⁸⁹Zr-trastuzumab avidity in the proximal left femur (arrow, SUVmax 7.7). (D) Biopsy of the proximal left femur demonstrated metastatic breast carcinoma with HER2 1+ on IHC (at 400X magnification), consistent with HER2-negative disease.

At the conclusion of Tasks 1-3, we had proven our initial hypotheses, that there is heterogeneity of HER2 expression in tumors, patients with HER2-negative primary malignancies may develop HER2-positive metastases, and non-invasive HER2-targeted imaging with ^{89}Zr -trastuzumab PET/CT could identify these patients. However, the false positive scans were a large limitation. By this time, there was evidence that pertuzumab, a newer monoclonal antibody to HER2, demonstrated greater specificity of HER2 binding than trastuzumab. We were able to produce a new HER2-targeted radiotracer, ^{89}Zr -pertuzumab, which was proven in animal models to accurately identify HER2-positive disease. At this time we petitioned the Department of Defense to allow us to stop further work with ^{89}Zr -trastuzumab, and perform a first-in-human study of ^{89}Zr -pertuzumab. If ^{89}Zr -pertuzumab could provide equal sensitivity for detection of HER2-positive disease, with better specificity, this would allow improvement in our protocol. **With the approval of the Department of Defense we initiated a first-in-human study of ^{89}Zr -pertuzumab PET/CT in patients with known HER2-positive disease, in order to demonstrate safety, dosimetry, and effectiveness of this radiotracer. This was then performed in Task 4.**

Task 4. Submission and approval of regulatory documents for ^{89}Zr -pertuzumab. Six-to-ten patient first-in-human study of ^{89}Zr -pertuzumab in patients with HER2+ breast cancer.

- 1a. IND for ^{89}Zr -trastuzumab
An IND for ^{89}Zr -trastuzumab was obtained
- 1b. IRB Protocol
An IRB Protocol for ^{89}Zr -trastuzumab was approved.
- 1c. Six-to-ten patient first-in-human study of ^{89}Zr -pertuzumab
Six patients were accrued to this portion of the protocol. This was adequate to demonstrate safety, dosimetry, and effectiveness of ^{89}Zr -pertuzumab to image HER2-positive disease. This portion of the protocol is complete as of 30-Sep-2017.

Task 4 has resulted in 6 patients completing the protocol with ^{89}Zr -pertuzumab PET/CT.

No toxicities occurred. Dosimetry estimates from Organ Level Internal Dose Assessment (OLINDA) demonstrated safe levels of radiation exposure from ^{89}Zr -pertuzumab. Multi-time point PET/CT demonstrated optimal imaging was achieved 5-8 days post-administration. ^{89}Zr -pertuzumab was able to image multiple sites of malignancy, and suggest they are HER2-positive. In two patients with both known HER2-positive and HER2-negative primary breast cancers and brain metastases, ^{89}Zr -pertuzumab PET/CT suggested the brain metastases were HER2-positive. In one of two patients, subsequent resection of a brain metastasis proved HER2-positive disease, confirming ^{89}Zr -pertuzumab-avidity was true positive for HER2-positive malignancy. A tabulated summary of the results from these 20 patients is described in Table 2.

Table 2: Patient Demographics and Study Results

Patient	Age	Gender	Known Sites of Malignancy at Time of ⁸⁹ Zr-pertuzumab Administration	Days Post- ⁸⁹ Zr-pertuzumab Administration of PET/CT Imaging	Sites of Demonstrably ⁸⁹ Zr-pertuzumab-avid Disease
1	46	Female	Brain	1, 2, 6, 8	Brain
2	68	Female	Nodal	1, 2, 5, 7	Nodal
3	58	Female	Breast, nodal	1, 2, 6, 7	Breast, nodal
4	69	Female	Liver, bone, chest wall	1, 4, 5, 8	Chest wall
5	38	Female	Brain, lung, nodal, liver	1, 2, 5	Brain, lung, nodal
6	42	Female	Nodal	1, 4, 5, 7	None

An example of successful HER2-targeted imaging with ⁸⁹Zr-pertuzumab is demonstrated in patient 1 (Figure 5). Patient 1 had two known primary breast malignancies, a right breast estrogen receptor (ER), progesterone receptor (PR), and HER2-positive (HER2 IHC 3+) IDC diagnosed in 2014 and the other ER, PR, and HER2-negative (HER2 IHC 0) IDC diagnosed in 2015. She had received HER2 directed therapy, including TDM-1 in 2015 and was currently on trastuzumab therapy at the time of the ⁸⁹Zr-pertuzumab PET/CT. She had a recent diagnosis of brain metastases. ⁸⁹Zr-pertuzumab PET/CT demonstrated progressive increase in ⁸⁹Zr-pertuzumab avidity over PET/CT scans obtained on days 1, 2, 6, and 8 following tracer administration (SUVmax of the most avid lesion was 13.6, 16.6, 26.0, and 30.1 on these four days; see Fig. 5A-D). Blood pool activity, including activity in the superior sagittal sinus, demonstrated continued decrease over the scans. This allowed most optimal visualization of the known brain metastases (Fig. 5E) on the day 8 scan (Fig. 5F).

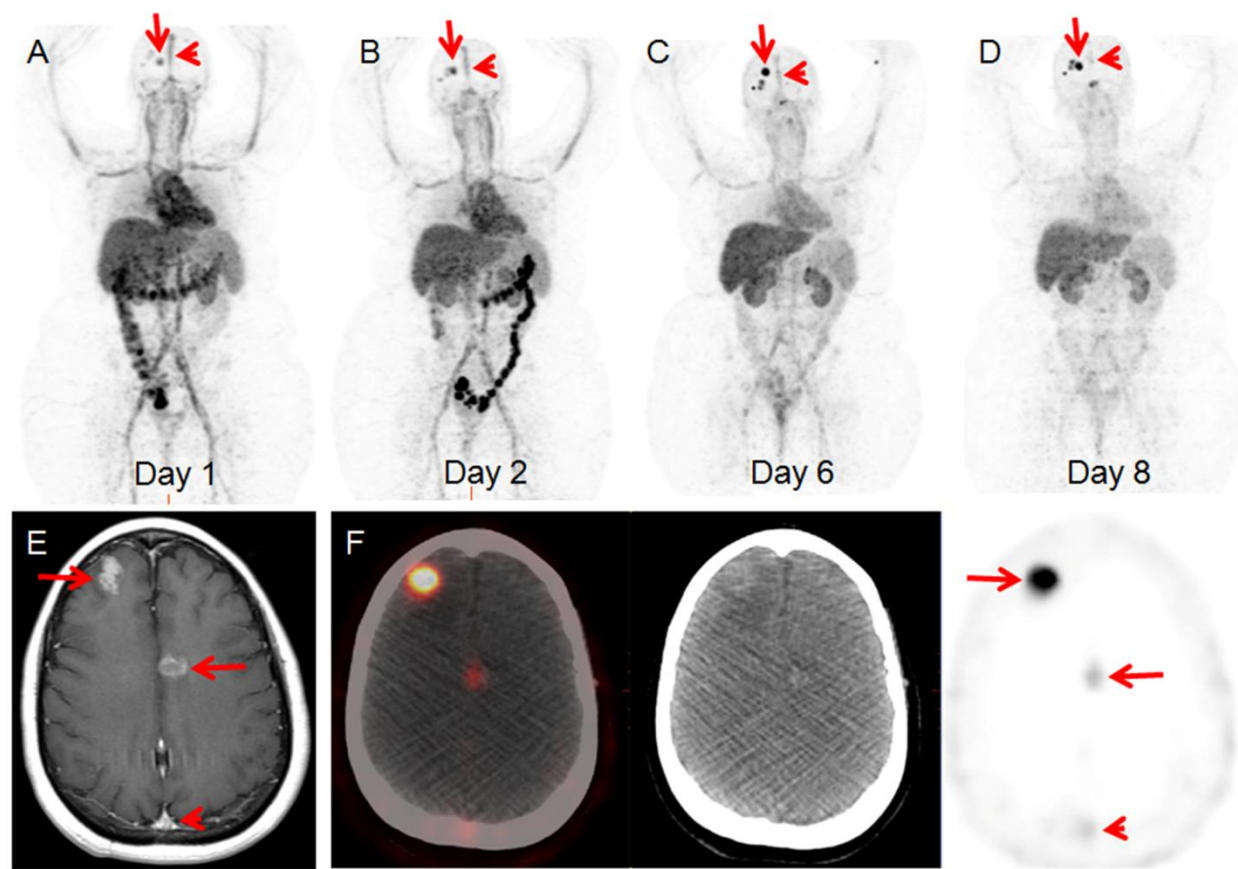


Figure 5: 46-year-old woman with both HER2-positive and HER2-negative primary breast malignancies and recently diagnosed brain metastases. Sequential maximum-intensity projection (MIP) images (A) 1 day, (B) 2 days, (C) 6 days, and (D) 8 days following administration of ^{89}Zr -pertuzumab. Blood pool and liver background clears on sequential images. Excreted bowel activity is seen on days 1 and 2. Bilateral kidney activity is visualized on all days. Increasing activity in foci overlying the skull is seen as time progresses (arrows). Decreasing activity is seen in the blood pool of the superior sagittal sinus (arrowheads). (E) Gadolinium-enhanced T1 weighed MR of the brain demonstrates enhancing brain metastases (arrows) and the superior sagittal sinus (arrowhead). (F) Axial fused PET/CT, CT, and PET images 8 days following ^{89}Zr -pertuzumab administration demonstrate avidity in the brain metastases (arrows) and minimal residual avidity in the superior sagittal sinus (arrowhead).

At the conclusion of Task 4, we had demonstrated first-in-human safety and effectiveness of ^{89}Zr -pertuzumab for imaging HER2-positive malignancy in patients with HER2-positive breast cancer. We now turn back to our initial goal of identifying HER2-positive malignancy in patients with presumed HER2-negative breast cancer. We will use ^{89}Zr -pertuzumab PET/CT in this patient population to determine if ^{89}Zr -pertuzumab PET/CT has equivalent ability to detect HER2-positive malignancy with fewer false positives than ^{89}Zr -trastuzumab PET/CT. This will be attempted in Tasks 5 and 6, which were being begun now.

Could HER2 heterogeneity open new therapeutic options in patients with HER2- primary breast cancer?

Task 5. Determine the proportion of patients with HER2- primary breast cancer who develop imagable HER2+ metastases using ^{89}Zr - pertuzumab

Performance of Task 5 is pending.

Task 6. Among patients with HER2+ metastases discovered in Task 5, determine if HER2 targeted therapy results in a measurable treatment response.

Performance of Task 6 is pending.

What opportunities for training and professional development has the project provided?

- Nothing to report

How were the results disseminated to communities of interest?

- Three manuscripts on the project have been published and are attached.
 1. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using the ^{89}Zr -DFO-trastuzumab PET/CT. Ulaner et al, Journal of Nuclear Medicine 2016, Oct;57(10)1523-1528. PMID: 27151988.
 2. ^{89}Zr -Trastuzumab PET/CT for detection of human epidermal growth factor receptor 2-positive metastases in patients with human epidermal growth factor receptor 2-negative primary breast cancer. Ulaner et al, Clinical Nuclear Medicine 2017, e-published ahead of print, PMID: 28872549.
 3. First-in-human HER2-targeted imaging using ^{89}Zr -pertuzumab PET/CT: Dosimetry and clinical application in patients with breast cancer. Journal of Nuclear Medicine 2017, Epub ahead of print. PMID: 29146695.
- Six presentations have been made including the project. One was a keynote address at an international conference.
 1. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using ^{89}Zr -DFO-trastuzumab. Presented by Gary Ulaner. Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging, San Diego, California, June 2016.

2. ^{89}Zr -Trastuzumab PET/CT for detection of human epidermal growth factor receptor 2-positive metastases in patients with human epidermal growth factor receptor 2-negative primary breast cancer. Presented by Gary Ulaner. Radiologic Society of North America 2017, Chicago, Illinois, December 2016.
3. Novel Radiotracers For Guiding Therapy for Patients with Breast Cancer, International **Keynote Speaker** for XII Professor Edwaldo Camargo Simpósio on PET/CT and SPECT/CT in Oncology. São Paulo, Brazil. May 2017.
4. Early phase positron emission tomography trials in patients with breast cancer. Presented by Gary Ulaner. Annual Meeting of the Society of Nuclear Meeting and Molecular Imaging, Denver, Colorado, June 2106.
5. FDG and Novel Radiotracers for Patients with Breast Cancer, Society of Nuclear Medicine and Molecular Imaging Mid Winter Meeting, Orlando, Florida, January 2018.
6. Incorporating Experimental Imaging Agents in Drug Clinical Trials. Medical Imaging in Clinical Research Conference, San Francisco, California, February 2018.

- **What do you plan to do during the next reporting period to accomplish the goals?**
 - Over the last year we have obtained an IND for ^{89}Zr -pertuzumab, obtained approval for an IRB protocol for with ^{89}Zr -pertuzumab imaging, and completed a first-in-human trial of ^{89}Zr -pertuzumab PET/CT in patients with HER2-positive metastatic breast cancer, demonstrating safety, dosimetry, and effectiveness of ^{89}Zr -pertuzumab for imaging HER2-positive disease. **We will now complete Tasks 5 and 6**, including the accrual of 21 patients with HER2-negative primary breast cancer, imaging with ^{89}Zr - pertuzumab to identify patients with foci suspicious for HER2-positive metastases, biopsies of there foci, and targeted HER2 therapy of any patients with newly proven HER2-positive malignancy.

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
 - The results already demonstrate the proof-of-concept that targeted imaging can be used to help identify patients eligible for targeted therapies.

- **What was the impact on other disciplines?**
 - The initial results confirm that there is heterogeneity of HER2 expression between the primary malignancy and distant metastases. This adds to the growing knowledge of tumor heterogeneity.
- **What was the impact on technology transfer?**
 - Nothing to report.
- **What was the impact on society beyond science and technology?**
 - Nothing to report.

5. CHANGES/PROBLEMS:

No current problems. ^{89}Zr -pertuzumab will now be used for imaging.

- **Changes in approach and reasons for change**
 - Nothing to report.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
 - Nothing to report.
- **Changes that had a significant impact on expenditures**
 - Nothing to report.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
 - Nothing to report.
- **Significant changes in use or care of human subjects**
 - Nothing to report.
- **Significant changes in use or care of vertebrate animals.**
 - Nothing to report.
- **Significant changes in use of biohazards and/or select agents**
 - Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

1. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using the ^{89}Zr -DFO-trastuzumab PET/CT. Ulaner et al,

Journal of Nuclear Medicine 2016, Oct;57(10)1523-1528. PMID: 27151988.

2. ⁸⁹Zr-Trastuzumab PET/CT for detection of human epidermal growth factor receptor 2-positive metastases in patients with human epidermal growth factor receptor 2-negative primary breast cancer. Ulaner et al, Clinical Nuclear Medicine 2017, e-published ahead of print, PMID: 28872549.

3. First-in-human HER2-targeted imaging using ⁸⁹Zr-pertuzumab PET/CT: Dosimetry and clinical application in patients with breast cancer. Journal of Nuclear Medicine 2017, Epub ahead of print. PMID: 29146695.

▪ **Books or other non-periodical, one-time publications.**

Nothing to report.

Other publications, conference papers, and presentations.

1. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using ⁸⁹Zr-DFO-trastuzumab. Presented by Gary Ulaner. Annual Meeting of the Society of Nuclear Medicine and Molecular Imaging, San Diego, California, June 2016.

2. ⁸⁹Zr-Trastuzumab PET/CT for detection of human epidermal growth factor receptor 2-positive metastases in patients with human epidermal growth factor receptor 2-negative primary breast cancer. Presented by Gary Ulaner. Radiologic Society of North America 2017, Chicago, Illinois, December 2016.

3. Novel Radiotracers For Guiding Therapy for Patients with Breast Cancer, International **Keynote Speaker** for XII Professor Edwaldo Camargo Simpósio on PET/CT and SPECT/CT in Oncology. São Paulo, Brazil. May 2017.

4. Early phase positron emission tomography trials in patients with breast cancer. Presented by Gary Ulaner. Annual Meeting of the Society of Nuclear Meeting and Molecular Imaging, Denver, Colorado, June 2106.

5. FDG and Novel Radiotracers for Patients with Breast Cancer, Society of Nuclear Medicine and Molecular Imaging Mid Winter Meeting, Orlando, Florida, January 2018.

6. Incorporating Experimental Imaging Agents in Drug Clinical Trials. Medical Imaging in Clinical Research Conference, San Francisco, California, February 2018.

- **Website(s) or other Internet site(s)**
Nothing to report.
 - **Technologies or techniques**
Nothing to report.
 - **Inventions, patent applications, and/or licenses**
Nothing to report.
 - **Other Products**
Nothing to report.
- **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
 - **What individuals have worked on the project?**
 - *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."*
- | | |
|-------|-----------------------------|
| Name: | Gary Ulaner (PI): No change |
|-------|-----------------------------|
- | | |
|-------|---|
| Name: | Hanh Pham (Research Assistant): No change |
|-------|---|
- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
 - Nothing to report
 - **What other organizations were involved as partners?**
 - Nothing to report

7. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** *Not applicable*
- **QUAD CHARTS:** *Not applicable.*

8. APPENDICES:

Three published manuscripts:

1. Detection of HER2-positive metastases in patients with HER2-negative primary breast cancer using the ^{89}Zr -DFO-trastuzumab PET/CT. Ulaner et al, Journal of Nuclear Medicine 2016, Oct;57(10)1523-1528. PMID: 27151988.
2. ^{89}Zr -Trastuzumab PET/CT for detection of human epidermal growth factor receptor 2-positive metastases in patients with human epidermal growth factor receptor 2-negative primary breast cancer. Ulaner et al, Clinical Nuclear Medicine 2017, e-published ahead of print, PMID: 28872549.
3. First-in-human HER2-targeted imaging using ^{89}Zr -pertuzumab PET/CT: Dosimetry and clinical application in patients with breast cancer. Journal of Nuclear Medicine 2017, Epub ahead of print. PMID: 29146695.

***** **ADDITIONAL NOTES:**

MARKING OF PROPRIETARY INFORMATION: Data that was developed partially or exclusively at private expense shall be marked as "Proprietary Data" and Distribution Statement B included on the cover page of the report. Federal government approval is required before including Distribution Statement B. The recipient/PI shall coordinate with the COR/GOR to obtain approval. **REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE.** It is the responsibility of the Principal Investigator to advise the COR/GOR when restricted limitation assigned to a document can be downgraded to "Approved for Public Release." **DO NOT USE THE WORD WHEN MARKING DOCUMENTS. DO NOT USE WATERMARKS WHEN MARKING DOCUMENTS.**

Detection of HER2-Positive Metastases in Patients with HER2-Negative Primary Breast Cancer Using ^{89}Zr -Trastuzumab PET/CT

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Our objective was to determine whether imaging with a human epidermal growth factor receptor 2 (HER2)-targeted PET tracer can detect HER2-positive metastases in patients with HER2-negative primary breast cancer. **Methods:** Patients with HER2-negative primary breast cancer and evidence of distant metastases were enrolled in an Institutional Review Board–approved prospective clinical trial. Archived pathologic samples from the patient's primary breast cancer were retested to confirm HER2-negative disease. Patients with confirmed HER2-negative primary breast cancer underwent ^{89}Zr -trastuzumab PET/CT to screen for ^{89}Zr -trastuzumab metastases. Metastases avid for ^{89}Zr -trastuzumab by PET/CT were biopsied and pathologically examined to define HER2 status. Patients with pathologically proven HER2-positive metastases subsequently received off-protocol HER2-targeted therapy to evaluate treatment response. **Results:** Nine patients were enrolled, all of whom had pathologic retesting that confirmed HER2-negative primary breast cancer. Five demonstrated suggestive foci on ^{89}Zr -trastuzumab PET/CT. Of these 5 patients, 2 had biopsy-proven HER2-positive metastases and went on to benefit from HER2-targeted therapy. In the other 3 patients, biopsy showed no evidence of HER2-positive disease, and their foci on ^{89}Zr -trastuzumab PET were considered false-positive. **Conclusion:** In this proof-of-concept study, we demonstrated that ^{89}Zr -trastuzumab PET/CT detects unsuspected HER2-positive metastases in patients with HER2-negative primary breast cancer. Although these are only initial results in a small sample, they are a proof of the concept that HER2-targeted imaging can identify additional candidates for HER2-targeted therapy. More specific HER2-targeted agents will be needed for clinical use.

Key Words: breast; molecular imaging; oncology; PET/CT; HER2; trastuzumab

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DOI: 10.2967/jnumed.115.172031

Human epidermal growth factor receptor 2 (HER2) is a critical biomarker in breast cancer, and its expression directly influences treatment. Approximately 20% of invasive ductal breast malignancies are classified as HER2-positive as a result of *ERBB2* gene amplification or the subsequent overexpression of the HER2 protein on the surface of tumor cells (1). Patients with HER2-positive breast cancer receive specific targeted HER2 therapies that reduce the risk of death, whereas patients with HER2-negative breast cancer do not receive them (2,3).

Heterogeneity of tumors both within and across lesions in a single patient is increasingly being documented, with significant therapeutic implications (4). Evidence from tissue samples suggests that HER2 expression may change between the primary breast malignancy and metastases (5–7). Inaccurate knowledge of receptor status in metastases due to tumor heterogeneity may lead to suboptimal selection of patients for HER2-targeted therapy. Indeed, data suggest that 10%–15% of patients with HER2-negative primary breast cancer may still benefit from HER2-targeted treatment (8). It is currently unclear why some patients with HER2-negative breast cancer may benefit from HER2-targeted treatments or how to identify them.

We hypothesized that some patients with HER2-negative primary malignancies develop HER2-positive metastases that can be identified by imaging. Such identification would be difficult by conventional biopsies, as only small samples from a limited number of lesions could be evaluated. In contrast, specific radiotracers that identify HER2 could allow a whole-body evaluation of all identifiable lesions. Specifically, ^{89}Zr -trastuzumab is a PET radiotracer that allows visualization of HER2-positive lesions (9). ^{89}Zr -trastuzumab PET/CT has been used in patients with known HER2-positive breast cancer to help determine which patients will respond to HER2-targeted therapy (10). We performed a prospective clinical trial evaluating the ability of ^{89}Zr -trastuzumab PET/CT to detect HER2-positive metastases in patients with HER2-negative primary breast cancer, and in this article we report the initial results of that trial.

MATERIALS AND METHODS

Patients

The study was performed under a prospective single-center protocol approved by the Institutional Review Board of Memorial Sloan

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Kettering Cancer Center (MSKCC) (ClinicalTrials.gov identifier NCT02286843). All patients provided written informed consent. Patients receiving treatment for metastatic HER2-negative primary breast cancer at MSKCC were identified as potential candidates. The inclusion criteria were a biopsy-proven HER2-negative primary malignancy, biopsy-proven metastatic disease, foci of demonstrable metastases on imaging modalities within 6 wk of enrollment, age greater than 18 y for women, and Eastern Cooperative Oncology Group performance score of 0–2. The exclusion criteria were a creatinine level higher than 2 times the upper limit of normal, an aspartate aminotransferase/alanine aminotransferase level higher than 2 times the upper limit of normal, life expectancy less than 3 mo, pregnancy or lactation, and inability to undergo PET/CT scanning because of weight limits.

Retesting of Archived Tissue

After receiving written consent from the patients, we retested their archived samples of primary breast cancer tissue to ensure that they were HER2-negative (Fig. 1). HER2 protein overexpression was evaluated by immunohistochemical staining using a Food and Drug Administration (FDA)-approved monoclonal antibody (clone 4B5; Ventana) directed against the internal domain of the c-erbB-2 oncoprotein (HER2). The immunohistochemistry scores were categorized according to the guidelines of the American Society of Clinical Oncology (ASCO) as follows: 0 or 1+, negative; 2+, equivocal; 3+, positive (Table 1) (11). Tissues with a score of 2+ were assessed for HER2 amplification with fluorescence in situ hybridization (FISH) in accord with the ASCO guidelines (11), using an FDA-approved probe set (*HER2* IQFISH pharmDx; Dako), and a positive FISH result was defined as a HER2/CEP17 (chromosome enumeration probe 17) ratio of at least 2.0. Tissues with an immunohistochemistry score of 0 or 1+ or an immunohistochemistry score of 2+ with a concurrently negative FISH result were classified as HER2-negative.

⁸⁹Zr-Trastuzumab PET/CT

After retesting of the archived tissue, patients with confirmed HER2-negative tumors underwent ⁸⁹Zr-trastuzumab PET/CT to assess for ⁸⁹Zr-trastuzumab foci suggestive of HER2-positive disease. ⁸⁹Zr-trastuzumab comprises the native HER2-targeted drug trastuzumab conjugated with desferoxamine and labeled with the positron-emitting metalloradionuclide ⁸⁹Zr, which has a half-life of 78 h, long enough to allow favorable biodistribution of radiolabeled intact antibodies. Trastuzumab is an FDA-approved monoclonal antibody that disrupts HER2 receptor signaling. MSKCC has an acknowledged investigational new drug (119907) from the FDA for human ⁸⁹Zr-trastuzumab imaging. ⁸⁹Zr-trastuzumab was produced under good-manufacturing-practice conditions: trastuzumab was

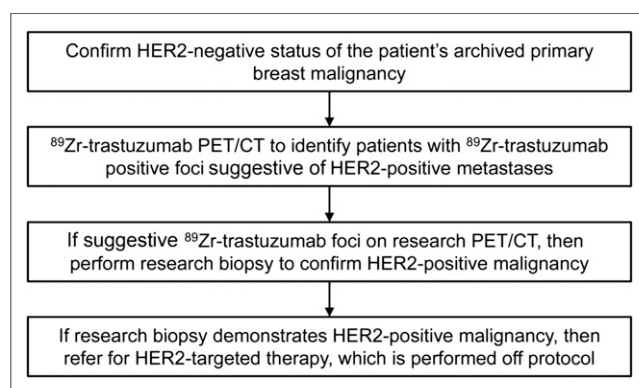


FIGURE 1. Protocol schema.

TABLE 1
Algorithm for Defining HER2 Expression in This Study

HER2 test result	Definition
Immunohistochemistry	
Score of 0 or 1+	Negative
Score of 2+	Equivocal
Score of 3+	Positive
FISH	
HER2/CEP17 ratio \geq 2.0	Positive
HER2/CEP17 ratio $<$ 2.0	Negative

First, immunohistochemistry is performed. If result is 2+, FISH is performed.

chelated with desferoxamine and subsequently radiolabeled with ⁸⁹Zr using a previously described methodology (12). The final drug product for human use was manufactured and quality control-tested by qualified personnel in conformance with the approved written standard operating procedures. The manufactured drug was tested before being released for patient administration to ensure that it conformed to the established acceptance specifications for appearance, pH, endotoxin content, residual solvent content, sterilizing filter integrity, radiochemical purity, and radiochemical identity. Sterility testing was initiated after the product had been released for patient administration.

Patients received a nominal 185 MBq \pm 10% of ⁸⁹Zr-trastuzumab intravenously over 5–10 min. To optimize tumor targeting, radiolabeled ⁸⁹Zr-trastuzumab was brought up to a final mass dose of 50 mg by the addition of nonradiolabeled trastuzumab at the end of the production (9). The final mass dose of 50 mg was provided by the MSKCC radiochemistry service to the clinic for patient administration. Five or six days after ⁸⁹Zr-trastuzumab administration, the patients underwent PET/CT from the mid-skull to the mid-thigh on a dedicated research Discovery PET/CT 710 scanner (GE Healthcare), with an 80-mA CT component for attenuation correction and lesion localization. The PET/CT images underwent iterative reconstruction, were displayed in multiplanar reconstructions, and were interpreted by 2 different nuclear medicine experts, both of whom were experienced in the use of novel research PET radiotracers. Physiologic ⁸⁹Zr-trastuzumab uptake was expected in the blood pool, liver, gallbladder, bowel, kidney, and (at a low grade) bone. Radiotracer uptake in areas that are not physiologic was graded both qualitatively and semiquantitatively. For qualitative grading, the foci were graded as suggestive or not suggestive. Only those foci qualitatively graded as suggestive by both interpreters were considered suspected lesions. For semiquantitative grading, 3-dimensional volumes of interest were placed around these suspected lesions, and the tracer uptake was graded using SUV_{max} (decay-corrected mean activity in volume of interest [μ Ci/ cm^3]/(injected dose [μ Ci)/body weight [g])).

Pathologic Confirmation

Image-guided biopsy of sites suggestive on PET/CT was performed in concert with an experienced oncologic interventional radiologist to minimize risk to the patient while obtaining high-quality samples. Biopsy specimens were evaluated by board-certified breast pathology specialists. Immunohistochemical staining was performed, and the results were categorized according to the ASCO guidelines (11) in the same way as for retesting of the archived

tissues. Specimens with an immunohistochemistry score of 3+ or with an immunohistochemistry score of 2+ and concurrently positive FISH results were classified as HER2-positive metastases. If the immunohistochemistry results were equivocal and FISH testing failed to produce a result after repeated attempts, the HER2 amplification status was also assessed using a hybrid capture-based next-generation sequencing assay, MSK-IMPACT (Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets), in a Clinical Laboratory Improvement Amendment–certified lab (13). This assay is designed to detect somatic genetic alterations in cancer-related genes, in addition to enabling the accurate assessment of genomewide copy number. Amplification of *ERBB2* is reported if the change is at least 2.0-fold on MSK-IMPACT.

Although therapy was not a component of this clinical trial, when HER2-positive metastases were identified and confirmed pathologically, this information was provided to the treating oncologists. HER2-targeted therapy was then initiated at the discretion of the treating oncologists per standard prescribing guidelines. Likewise, imaging to determine tumor response to HER2-targeted therapy was not defined by the protocol.

RESULTS

Patient Characteristics

Between December 2014 and July 2015, 9 patients, all women with estrogen receptor (ER)–positive and HER2-negative primary invasive ductal breast cancer, completed the study protocol. The patient characteristics are summarized in Table 2.

Metastatic Sites at Enrollment

All patients had at least one site of metastatic disease proven by biopsy. Once one site of metastatic disease was proven by biopsy, additional sites were determined from abnormalities on contrast-enhanced CT or ¹⁸F-FDG PET/CT. The most common sites of distant metastases at the time of enrollment were nodes ($n = 8$), followed by bone ($n = 7$), liver ($n = 7$), lung ($n = 2$), adrenal gland ($n = 1$), and pleura ($n = 1$). Eight of 9 patients had metastatic involvement in multiple organ systems.

Retesting of Archived Tissue

On immunohistochemical retesting of the patients' archived primary breast cancer specimens, all 9 patients had confirmed HER2-negative primary malignancies. Four patients had an immunohistochemistry score of 0, whereas the other 5 had an immunohistochemistry score of 1+.

⁸⁹Zr-Trastuzumab PET/CT

All 9 patients underwent ⁸⁹Zr-trastuzumab PET/CT. They were monitored for side effects for 30 min after tracer injection, as well as being telephoned the following day, and no side effects were observed or reported. Vital signs were recorded before and after injection, and there were no changes that had a clinical impact.

In 5 of the 9 patients (56%), both interpreters observed foci of ⁸⁹Zr-trastuzumab avidity considered suggestive of HER2-positive disease. In no case were the suggestive foci seen by only one interpreter. Both interpreters were able to compare the ⁸⁹Zr-trastuzumab PET/CT results with prior imaging studies. In 3 patients, the suggestive organ system was bone, whereas one patient exhibited suggestive nodal foci, and another had a suggestive adrenal focus.

Pathologic Confirmation

Image-guided biopsy was performed on 5 patients with suggestive ⁸⁹Zr-trastuzumab foci.

Patient 2 was a 41-y-old woman who, in March 2014, had undergone a right mastectomy with pathologically demonstrated ER-positive/HER2-negative primary invasive ductal carcinoma (Fig. 2A). Thus, she was treated initially with ovarian suppression and tamoxifen and then with fulvestrant and aromatase inhibition, but the response to treatment was mixed. In November 2014, CT demonstrated enlarged thoracic nodes consistent with malignancy, and in December 2014, ⁸⁹Zr-trastuzumab PET/CT demonstrated ⁸⁹Zr-trastuzumab-avid thoracic nodes (Fig. 2B). The most avid was a right supraclavicular node (SUV_{max}, 4.6), which underwent biopsy and demonstrated an immunohistochemistry score of 3+ (Fig. 2C). This ⁸⁹Zr-trastuzumab focus was considered true-positive for HER2-positive distant metastasis. The patient was then switched to treatment with

TABLE 2
Patient Demographics and HER2 Expression Results

Patient no.	Age (y)	Metastatic sites at enrollment	Confirmatory HER2 IHC of primary breast cancer	Suggestive foci on ⁸⁹ Zr-trastuzumab PET/CT?	Image-guided biopsy results
1	46	Bone, liver	0	None	
2	41	Nodes	1+	Nodes (SUV _{max} , 4.6)	IHC, 3+
3	58	Bone, liver, nodes	0	Bone (SUV _{max} , 7.1)	IHC, 1+
4	69	Bone, liver, nodes	0	None	
5	38	Bone, liver, nodes	1+	Bone (SUV _{max} , 5.9)	IHC, 2+; FISH, failure; MSK-IMPACT, amplified
6	42	Nodes, adrenal	1+	Adrenal (SUV _{max} , 9.2)	IHC, 2+; FISH, 1.4
7	83	Bone, liver, nodes, lung	1+	Bone (SUV _{max} , 9.7)	IHC, 1+
8	54	Bone, liver, nodes, lung	0	None	
9	48	Bone, liver, nodes, pleura	1+	None	

IHC = immunohistochemistry score.

All 9 women had primary invasive ductal breast cancer.

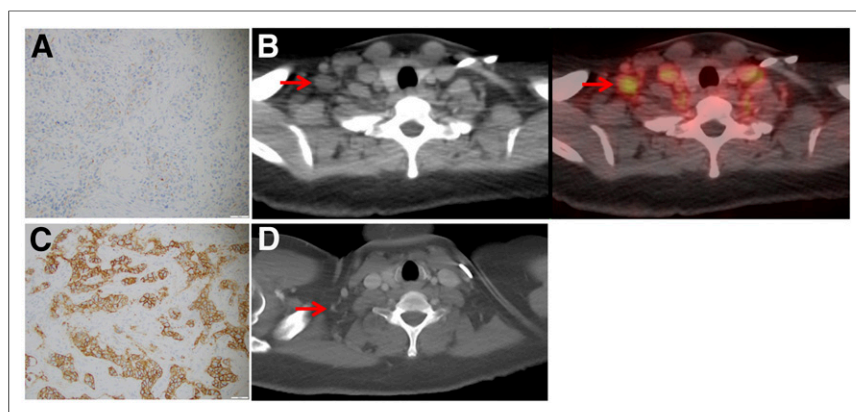


FIGURE 2. 41-y-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma and recurrence in thoracic nodes. (A) Immunohistochemistry score of primary breast malignancy was 1+ (at $\times 20$ magnification), consistent with HER2-negative malignancy. (B) Axial CT and ^{89}Zr -trastuzumab PET/CT demonstrated ^{89}Zr -trastuzumab avidity in enlarged right supraclavicular nodes (arrows, SUV_{max} of 4.6) and left internal mammary nodes (not shown). (C) Biopsy of right supraclavicular node demonstrated metastatic breast carcinoma with immunohistochemistry score of 3+ (at $\times 20$ magnification), consistent with HER2-positive disease. Patient began systemic treatment including trastuzumab and pertuzumab. (D) Follow-up axial CT after 2 mo of treatment demonstrated resolution of nodes on CT (arrow).

trastuzumab, pertuzumab, and paclitaxel. Follow-up CT of the chest, abdomen, and pelvis demonstrated resolution of the previously enlarged lymph nodes and absence of new lesions, consistent with a complete response (Fig. 2D). As of November 2015, the patient remained on trastuzumab and pertuzumab alone and was without evidence of disease.

Patient 5 was a 38-y-old woman who, in July 2011, had undergone a left mastectomy with pathologically demonstrated ER-positive/HER2-negative primary invasive ductal carcinoma (Fig. 3A). Osseous, hepatic, and nodal metastases were identified beginning in October 2013 and were progressing despite systemic

therapy with fulvestrant, leuprolide, and a novel clinical trial therapeutic. In April 2015, ^{89}Zr -trastuzumab PET/CT demonstrated multiple suggestive osseous foci (Fig. 3B). The most avid foci were in the right ilium and right proximal femur (SUV_{max} , 5.9). Biopsy of the right ilium demonstrated equivocal HER2 findings. The immunohistochemistry results were equivocal (Fig. 3C) because of incomplete membranous staining in a small percentage of the cells. FISH failed to produce a result after repeated attempts. Given the equivocal results, further testing was performed with the MSK-IMPACT assay (13). The change in *ERBB2* on MSK-IMPACT was 2.6-fold (Fig. 3D). Therefore, this ^{89}Zr -trastuzumab focus was considered true-positive for a HER2-positive distant metastasis (14). The patient was then switched to treatment with trastuzumab, pertuzumab, and docetaxel and was followed up with ^{18}F -FDG PET/CT, which showed a decrease in the size and ^{18}F -FDG avidity of the liver and nodal metastases, as well as a decrease in the

^{18}F -FDG avidity of the osseous lesions, representing a partial response to treatment (Figs. 4A and 4B). In the liver, multiple ^{18}F -FDG-avid lesions resolved after treatment, whereas a residual lesion in segment 4 showed an SUV_{max} decrease from 8.2 to 5.6. In the osseous system, multiple ^{18}F -FDG-avid lesions resolved after treatment and others showed an SUV_{max} decrease, such as a decrease from 9.1 to 4.7 in the body of T11.

Patients 3, 6, and 7 had suggestive foci on ^{89}Zr -trastuzumab PET/CT, but the pathologic findings from image-guided biopsy specimens were consistent with HER2-negative metastatic breast cancer (Table 2). For example, patient 7 was an 83-y-old woman

who presented in June 2010 with metastatic ER-positive, HER2-negative invasive ductal breast cancer (Fig. 5A). Despite several courses of chemotherapy, in 2015 she had persistent osseous, hepatic, nodal, and pulmonary metastases on ^{18}F -FDG PET/CT. ^{89}Zr -trastuzumab PET/CT was performed in July 2015 and demonstrated multiple suggestive osseous foci (Figs. 4B and 4C). Because the most avid osseous lesion was in the cervical spine (SUV_{max} , 9.7), a more easily assessable lesion in the proximal left femur (SUV_{max} , 7.7) was chosen for biopsy. Pathologic examination demonstrated metastatic breast cancer, but the immunohistochemistry score was only 1+, consistent with HER2-negative disease (Fig. 4D). This ^{89}Zr -trastuzumab focus was considered a false-positive finding. Similarly, biopsy found that the foci in the left ilium of patient 3 were false-positive (SUV_{max} , 7.1), as were the foci in the left adrenal gland of patient 6 (SUV_{max} , 9.2).

Patients 1, 4, 8, and 9 did not have suggestive ^{89}Zr -trastuzumab foci.

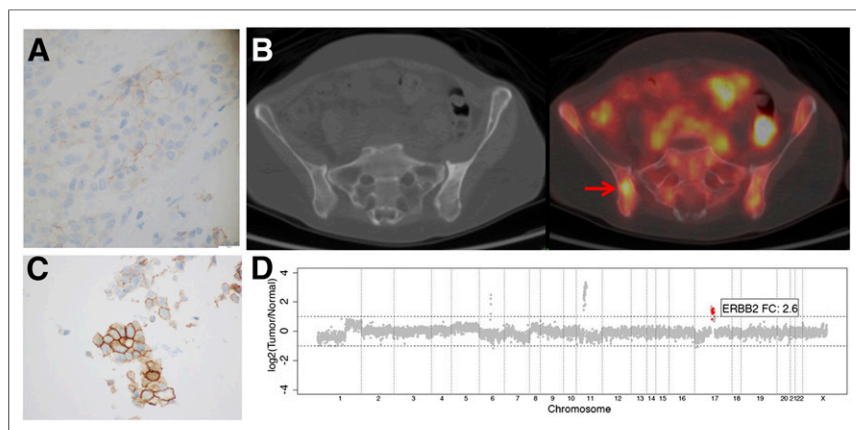


FIGURE 3. 38-year-old woman with primary ER-positive/HER2-negative invasive ductal breast carcinoma. (A) Immunohistochemistry score of primary breast malignancy was 1+ (at $\times 400$ magnification), consistent with HER2-negative malignancy. (B) Axial CT and ^{89}Zr -trastuzumab PET/CT demonstrated ^{89}Zr -trastuzumab avidity in right ilium (arrow, SUV_{max} of 5.9). Avidity in bowel is considered physiologic. (C) Biopsy of right ilium demonstrated metastatic breast carcinoma with equivocal immunohistochemistry score of 2+ (at $\times 400$ magnification). (D) MSK-IMPACT copy-number plot demonstrating HER2 amplification. Each dot represents probe set, and values on y-axis show log₂-transformed ratio of tumor vs. normal. *ERBB2* change was 2.6-fold, consistent with HER2-positive disease.

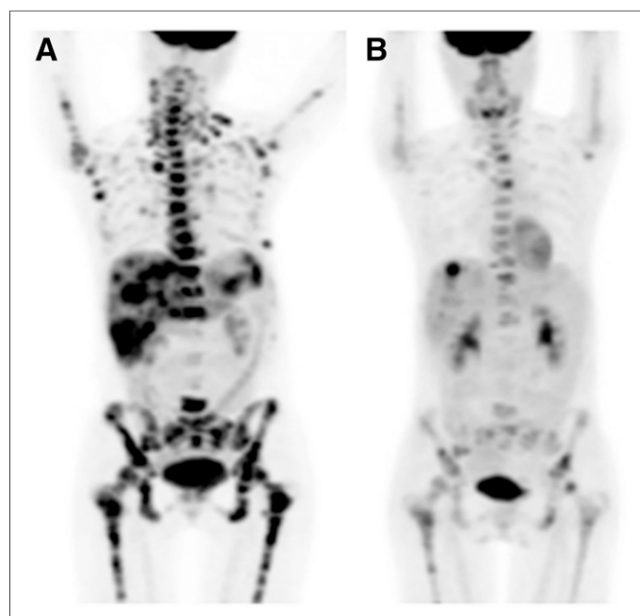


FIGURE 4. Patient from Figure 3 underwent HER2-targeted therapy after biopsy had demonstrated HER2 amplification in osseous metastasis. Maximum-intensity projections from ^{18}F -FDG PET/CT studies before (A) and after (B) 3 mo of systemic treatment including trastuzumab and pertuzumab demonstrate treatment response.

DISCUSSION

Heterogeneity within a tumor, as well as across multiple tumors within a patient, has often been demonstrated. Thus, a limited number of small biopsies may not be able to accurately characterize multiple tumors in a single patient. This limitation has substantial implications for patients with breast cancer, which critically requires accurate documentation of receptor phenotype for selection of targeted systemic therapies. In this study, we demonstrated a proof of the concept that targeted HER2 imaging can detect unsuspected HER2-positive metastases in patients initially classified as having a HER2-negative primary breast malignancy. We also showed that these patients may go on to benefit from HER2-targeted therapy.

Although medical imaging of oncology patients has traditionally been used to detect tumors, determine stage, and evaluate treatment response, this study suggests that targeted medical imaging may help physicians select the particular targeted systemic therapy from which an individual patient can benefit. Over 900,000 women are currently living with metastatic breast cancer, with more than 50,000 new cases diagnosed each year (15). Eighty percent of these women have HER2-negative primary malignancies. If just 10% of the patients with metastatic HER2-negative primary breast cancer were found to harbor HER2-positive metastases, that would represent a current population of over 72,000 women. Thus, targeted HER2 imaging could substantially increase the number of patients who may be eligible for and benefit from HER2-targeted therapies.

Of the initial 9 patients with HER2-negative primary breast cancer in our study, 2 patients had suggestive ^{89}Zr -trastuzumab foci that were classified as HER2-positive metastases. One of these patients had a primary malignancy with a negative immunohistochemistry result and a metastasis with a positive immunohistochemistry result. The other patient had a primary malignancy with a negative immunohistochemistry result but a metastasis with an equivocal result. Use of the MSK-IMPACT assay in this patient after failure of

FISH testing was positive for *ERBB2* amplification. Detection of *ERBB2* amplification by next-generation sequencing is not currently part of the ASCO guidelines (13); however, MSK-IMPACT detection of copy-number alterations correlates strongly with immunohistochemistry and FISH and thus, in this study, was considered evidence of HER2 positivity (14). A response to systemic HER2-targeted therapy was demonstrated in 2 patients, but because these patients also received concomitant taxane antimetabolic chemotherapy, a definitive conclusion cannot be drawn about the HER2 therapy. Confirming the efficacy of HER2-targeted therapy in patients identified as eligible by HER2-targeted imaging will require further study.

Three patients had suggestive ^{89}Zr -trastuzumab foci that on biopsy were classified as HER2-negative metastases. Two of these patients underwent osseous biopsy demonstrating an immunohistochemistry score of only 1+. The third patient underwent an adrenal biopsy demonstrating an immunohistochemistry score of 2+ and a concurrent FISH ratio of 1.4, leading to classification as HER2-negative. Because only one site was biopsied in each patient, it was not proven that all ^{89}Zr -trastuzumab foci were negative on pathology. Of course, for ethical and logistic reasons it would not be possible to biopsy all ^{89}Zr -trastuzumab foci in a patient; thus, the available results from the biopsied sites were used to classify patients.

The explanation for ^{89}Zr -trastuzumab foci in lesions without high levels of HER2 expression by immunohistochemistry or FISH is still unknown. One possibility is that decalcification of osseous lesions may decrease the intensity of immunohistochemical staining, resulting in a false-negative pathologic result (16). This possibility may have accounted for the osseous biopsy results in this study but would

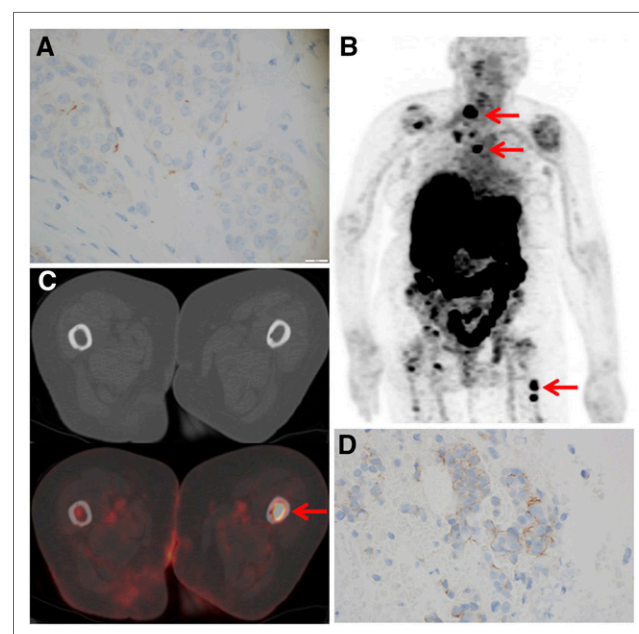


FIGURE 5. 83-y-old woman with primary ER-positive/HER2-negative invasive ductal breast carcinoma. (A) Immunohistochemistry score of primary breast malignancy was 1+ (at $\times 400$ magnification), consistent with HER2-negative malignancy. (B) ^{89}Zr -trastuzumab maximum-intensity projection demonstrates several foci of ^{89}Zr -trastuzumab avidity that localize to osseous structures. Avidity in liver and bowel is considered physiologic. (C) Axial CT and ^{89}Zr -trastuzumab PET/CT demonstrate ^{89}Zr -trastuzumab avidity in proximal left femur (arrow, SUV_{max} of 7.7). (D) Biopsy of proximal left femur demonstrated metastatic breast carcinoma with immunohistochemistry score of 1+ (at $\times 400$ magnification), consistent with HER2-negative disease.

not affect the adrenal biopsy result. Another possible explanation is release of free ^{89}Zr from its chelator during the long 5-d uptake period. When loosely chelated, ^{89}Zr is known to be a bone seeker (17). Indeed, evidence suggests that when ^{89}Zr is chelated to antibodies with desferoxamine, radioactivity accumulates in the bone (18). Thus, nonspecific binding of potentially free ^{89}Zr at sites of osseous turnover associated with bone metastases could be the reason for the false-positive osseous foci in this study. Nonetheless, patients 1, 4, 8, and 9, who had metastatic bone disease, were negative on ^{89}Zr -trastuzumab imaging. The high HER2 expression based on ^{89}Zr -trastuzumab imaging could be related to in vivo internalization rates or affinity differences that would not necessarily be reflected by immunohistochemistry or FISH (19). The development of more specific radiotracers to reduce false-positive foci on PET may be important. Potential alternatives include HER2-targeting Affibody molecules (20) and Nanobodies (21,22), which have the advantage of a rapid biodistribution that allows imaging within hours of tracer administration, rather than days as required after antibody tracers. The advantage of Affibody molecules and Nanobodies would be more pronounced if multiple scans were performed on a single patient, such as if there were a need to assess the response of HER2-positive disease at multiple time-points. Affibody molecules and Nanobodies labeled with shorter-half-life tracers may result in a lower radiation dose to patients and can often be imaged on the day they are injected, resulting in fewer patient visits.

Although this study had a limited sample, it is interesting to note that the intensity of ^{89}Zr -trastuzumab uptake did not correlate with the intensity of HER2 expression on immunohistochemistry. Indeed, the sites of highest ^{89}Zr -trastuzumab avidity were the false-positives, whereas the patient with the strongest immunohistochemistry score, 3+, demonstrated only moderate ^{89}Zr -trastuzumab uptake. The reason for this finding is currently unknown.

The strength of this study was its design as a prospective clinical trial, whereas its weakness was the relatively small sample size. Ethical and logistic reasons prevent biopsy of all ^{89}Zr -trastuzumab foci; thus, the patients had their pathology classified from a limited number of biopsies. Likewise, ethical reasons prevent biopsy confirmation of the HER2-negative status of metastases in patients without suggestive foci on ^{89}Zr -trastuzumab. This paper is presented as a proof of concept, not a demonstration of the accuracy of ^{89}Zr -trastuzumab for imaging HER2-positive and -negative metastases. These preliminary results are encouraging, but larger studies will be needed to further evaluate the value of ^{89}Zr -trastuzumab for the imaging of HER2-positive breast cancer.

CONCLUSION

^{89}Zr -trastuzumab PET/CT detects unsuspected HER2-positive metastases in patients with HER2-negative primary breast cancer and thus identifies patients who are eligible for highly effective HER2-targeted therapies but would otherwise be overlooked by conventional means. Although these are only initial results from a small sample, the study is a proof of the concept that targeted imaging may help identify patients with actionable targets. More specific HER2-targeted agents will be needed for clinical utility.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with

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Detection of HER2-Positive Metastases in Patients with HER2-Negative Primary Breast Cancer Using ^{89}Zr -Trastuzumab PET/CT

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⁸⁹Zr-Trastuzumab PET/CT for Detection of Human Epidermal Growth Factor Receptor 2–Positive Metastases in Patients With Human Epidermal Growth Factor Receptor 2–Negative Primary Breast Cancer

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Purpose: The aim of this study was to determine if imaging with ⁸⁹Zr-trastuzumab, a human epidermal growth factor receptor 2 (HER2)–targeting PET tracer, can detect HER2-positive metastases in patients with HER2-negative primary breast cancer.

Methods: As part of an institutional review board–approved, prospective clinical trial of ⁸⁹Zr-trastuzumab PET/CT (ClinicalTrials.gov identifier NCT02286843), a second group of 11 patients with HER2-negative primary breast cancer and known metastatic disease were recruited. Patients with confirmed HER2-negative primary breast cancer underwent ⁸⁹Zr-trastuzumab PET/CT to screen for ⁸⁹Zr-trastuzumab–avid lesions suggestive of unsuspected HER2-positive metastases. ⁸⁹Zr-trastuzumab–avid lesions on PET/CT were biopsied and pathologically examined to determine HER2 status.

Results: All 11 patients had confirmed HER2-negative primary breast cancer. Four patients demonstrated suspicious foci on ⁸⁹Zr-trastuzumab PET/CT. Of these 4 patients, 1 patient had biopsy-proven HER2-positive metastases. The other 3 patients with suspicious ⁸⁹Zr-trastuzumab–avid foci had biopsy demonstrating a metastasis that was HER2-negative and were considered false-positive ⁸⁹Zr-trastuzumab PET foci. Combined with a published report of the first 9 patients, there have been a total of 20 HER2-negative primary breast cancer patients, with 3 patients (15%) having pathologically confirmed HER2-positive distant metastases and 6 (30%) with suspicious ⁸⁹Zr-trastuzumab–avid foci that were HER2-negative on pathology, which were thus considered false-positive ⁸⁹Zr-trastuzumab findings.

Conclusions: This second group of patients confirms the proof of concept that ⁸⁹Zr-trastuzumab PET/CT detects unsuspected HER2-positive metastases in a subset of patients with HER2-negative primary breast cancer. False-positive ⁸⁹Zr-trastuzumab–avid foci present a challenge to using this tracer.

Key Words: breast cancer, HER2, PET/CT, trastuzumab

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Approximately 20% of invasive ductal breast malignancies are classified as human epidermal growth factor receptor 2 (HER2)–positive because of overexpression of the HER2 protein

on the surface of tumor cells or gene amplification.¹ Classification of a breast malignancy as HER2-positive or HER2-negative is an essential component of clinical care, as patients with HER2-positive breast cancer receive specific targeted HER2 therapies that reduce the risk of death, whereas patients with HER2-negative breast cancer do not.^{2,3} An important question is whether HER2 status could be heterogeneous between sites of malignancy in a single patient. If heterogeneity exists, then classification of HER2 status from 1 biopsy may inaccurately describe the extent of disease in a patient with the potential to respond to HER2-targeted therapy. Indeed, heterogeneity of tumors both within and across lesions in a single patient is increasingly being recognized.⁴ Investigators have identified patients with HER2-positive primary malignancies in which metastases have lost HER2 positivity.⁵ Potentially more clinically significant, investigators have identified patients with HER2-negative primary malignancies in which metastases have acquired HER2 positivity. These patients may benefit from HER2-targeted therapies that they are not receiving because they are not considered to have HER2-positive disease.

It is currently unclear how to best identify patients who have malignancy with heterogeneous HER2 expression. Identification by biopsy would be difficult because only small samples of tissue from a limited number of lesions are normally sampled. We hypothesized that HER2 heterogeneity may be identified by imaging with a HER2-targeting radiotracer and designed a prospective clinical trial to evaluate HER2 heterogeneity with ⁸⁹Zr-trastuzumab PET/CT, which allows for visualization of HER2-positive malignancy.⁶ We published the initial results from the first 9 patients and demonstrated the ability of ⁸⁹Zr-trastuzumab PET/CT to identify HER2-positive metastases in patients with HER2-negative primary breast malignancy.⁷ We present the results of the second group of patients, comprising 11 patients.

PATIENTS AND METHODS

Patients

A single-center, prospective, institutional review board–approved protocol (ClinicalTrials.gov identifier NCT02286843) was performed. Patients receiving treatment for metastatic HER2-negative primary breast cancer were identified as potential candidates. Inclusion criteria were as follows: (1) biopsy-proven HER2-negative primary malignancy; (2) biopsy-proven metastatic disease; (3) foci of demonstrable metastases on imaging modalities within 6 weeks of enrollment; (4) women 18 or older; and (5) Eastern Cooperative Oncology Group performance score of 0 to 2. Exclusion criteria were as follows: (1) creatinine more than 2 times the upper limit of normal; (2) aspartate aminotransferase/alanine aminotransferase more than 2 times the upper limit of normal; (3) life expectancy of less than 3 months; (4) pregnancy or lactation; and (5) inability

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TABLE 1. Algorithm for Defining HER2 Expression*

First, HER2 testing is performed with IHC. IHC results:	
0 or 1+	HER2-negative
2+	Equivocal
3+	HER2-positive
If IHC is 2+ (equivocal), then dual-probe HER2 FISH is performed. FISH results:	
HER2/CEP17 ratio ≥2.0	HER2-positive
HER2/CEP17 ratio <2.0	HER2-negative
*As per ASCO guidelines. ⁸	
CEP17 indicates chromosome enumeration probe 17.	

to undergo PET/CT scanning because of weight limits. All patients enrolled in the protocol provided written informed consent.

Confirmation of HER2 Status of the Primary Breast Malignancy

The primary breast cancer tissue sample for each patient was retested for HER2 status to ensure proper classification as HER2-negative. Human epidermal growth factor receptor 2 protein overexpression was evaluated by immunohistochemistry (IHC) using a US Food and Drug Administration (FDA)–approved monoclonal antibody (clone 4B5; Ventana, Tucson, AZ) directed against the internal domain of the c-erbB-2 oncoprotein (HER2). The IHC results were categorized as follows: 0, 1+ = negative result, 2+ = equivocal result, and 3+ = positive result, according to the published American Society of Clinical Oncology (ASCO) guidelines⁸ (Table 1). Tissues with 2+ staining (equivocal) were assessed for HER2 amplification with fluorescence in situ hybridization (FISH) as per ASCO guidelines⁸ using an FDA-approved probe set (*HER2* IQFISH pharmDx; Dako, Pittsburgh, PA) and defined as HER2/CEP17 ratio of 2.0 or greater. Carcinoma with 0 or 1+ IHC staining or 2+ IHC staining and concurrent negative HER2 FISH (HER2/CEP17 ratio <2.0) were classified as HER2-negative primary breast malignancies.

⁸⁹Zr-Trastuzumab PET/CT

Patients with confirmed HER2-negative primary breast malignancy underwent ⁸⁹Zr-trastuzumab PET/CT. ⁸⁹Zr-trastuzumab

is composed of the native trastuzumab, an FDA-approved HER2-targeting monoclonal antibody, conjugated with desferrioxamine and labeled with the positron-emitting metalloradionuclide ⁸⁹Zr. ⁸⁹Zr has a half-life of 78 hours, long enough to allow favorable biodistribution of radiolabeled intact antibodies. Our institution has an FDA Investigational New Drug for human ⁸⁹Zr-trastuzumab imaging (Memorial Sloan Kettering Cancer Center Investigational New Drug no. 119907). ⁸⁹Zr-trastuzumab was produced under Good Manufacturing Practice conditions using previously described methodology.⁹ The final drug product for human use was manufactured, and quality control tested prior to being released for patient administration to ensure that it conformed to the established acceptance specifications for appearance, pH, endotoxin content, sterilizing filter integrity, radiochemical purity, and radionuclidic identity. Sterility and immunoreactivity determinations were performed after release.

Patients were intravenously administered 185 MBq ± 10% of ⁸⁹Zr-trastuzumab over 5 minutes. Radiolabeled ⁸⁹Zr-trastuzumab was brought up to a final mass dose of 50 mg by adding non-radiolabeled trastuzumab at the end of the production because this has been shown to help optimize tumor targeting.⁶ The final mass dose of 50 mg was provided by the Radiochemistry and Molecular Imaging Probe Core at Memorial Sloan Kettering Cancer Center to the clinic for patient administration. Five or 6 days following ⁸⁹Zr-trastuzumab administration, patients underwent PET/CT from the midskull to midthigh on a dedicated research GE Discovery PET/CT 710 scanner (GE Healthcare, Chicago, IL), with an 80-mA CT component for attenuation correction and lesion localization. PET/CT images were reconstructed using iterative reconstruction and displayed in multiplanar reconstructions. ⁸⁹Zr-trastuzumab PET/CT scans were interpreted by 2 different nuclear medicine experts (G.A.U., J.A.C.), both experienced in the use of PET antibody radiotracers. Both readers were able to compare ⁸⁹Zr-trastuzumab PET/CT results with prior imaging studies. Physiologic ⁸⁹Zr-trastuzumab uptake was expected in the blood pool, liver, gallbladder, bowel, and kidney. Radiotracer uptake in areas that are not physiologic were graded both qualitatively and semiquantitatively. For qualitative scoring, lesions were scored as suspicious or not suspicious. Only those foci qualitatively scored as suspicious by both readers were considered suspicious foci. Semiquantitative analysis of tracer uptake was performed by recording the SUVmax of suspicious lesions. Three-dimensional volumes of interest were placed

TABLE 2. Patient Demographics and Study Results

Patient	Age, y	Sites of Distant Metastases at Enrollment	Confirmatory IHC of Primary Breast Cancer	Suspicious Foci on ⁸⁹ Zr-Trastuzumab PET/CT	Image-Guided Biopsy Results
10	54	Bone, nodes	HER2 1+	None	
11	40	Bone, liver, nodes, chest wall	HER2 0	Bone (SUV 10.1) and nodal (SUV 5.1)	HER2 IHC 2+, FISH 1.6
12	56	Liver	HER2 1+	None	
13	43	Nodes, lung	HER2 1+	None	
14	47	Liver, nodes, pleura	HER2 2+ FISH 1.4	Liver (SUV 10.9) and pleura (SUV 6.9)	HER2 IHC 2+, FISH 2.4
15	50	Bone, liver	HER2 1+	None	
16	49	Bone, liver	HER2 0	None	
17	51	Bone, nodes, pleura	HER2 0	Lung (SUV 11.3)	HER2 1+
18	48	Bone, liver, nodes	HER2 1+	None	
19	60	Liver	HER2 1+	None	
20	58	Bone, liver, nodes	HER2 1+	Liver (SUV 15.7)	HER2 1+

Patients 11 to 20, all with primary invasive ductal breast cancer, are reported here. The first 9 patients were reported in the initial results of the trial.⁷

in these areas, and tracer uptake was quantified using SUV, calculated as decay-corrected mean region of interest activity ($\mu\text{Ci}/\text{mL}$)/(injected dose (μCi)/body weight (g)).

Biopsy and Pathologic Evaluation of Suspicious ^{89}Zr -Trastuzumab Foci

Image-guided biopsy was selected in concert with an experienced oncologic interventional radiologist to minimize risks to the patient while obtaining high-quality samples. Biopsy specimens were evaluated by board-certified breast pathology specialists. The IHC results were categorized according to published ASCO guidelines,⁸ as discussed previously and in Table 1. Carcinomas with 3+ IHC staining or 2+ IHC staining and concurrent positive HER2 FISH were classified as HER2-positive metastases.

RESULTS

Patient Characteristics

Between December 2015 and May 2016, a second group of 11 women with HER2-negative primary invasive ductal breast cancer completed the study protocol. These were designated patient numbers 10 to 20, following the first 9 patients described in the

initial publication.⁷ Patient characteristics and study results are summarized in Table 2.

Sites of Distant Metastases at Time of Protocol Enrollment

All 11 patients had at least 1 site of metastatic disease at the time of protocol enrollment. The most common sites of distant metastases were bone ($n = 7$), nodal ($n = 7$), and liver ($n = 7$), followed by lung ($n = 2$), pleural ($n = 1$), and chest wall ($n = 1$). Nine of the 11 patients had multiple organ system metastatic involvement.

Confirmation of HER2 Status of the Primary Breast Malignancy

On IHC retesting of the patients' archived primary breast cancer specimens, all 11 patients had confirmed HER2-negative primary malignancies according to published ASCO guidelines.⁸ Three patients had HER2 IHC scores of 0, whereas 7 had HER2 IHC scores of 1+, and 1 had a HER2 IHC score of 2+ with a FISH of 1.4.

^{89}Zr -Trastuzumab PET/CT

All 11 patients underwent ^{89}Zr -trastuzumab PET/CT. Patients were monitored for 30 minutes after tracer injection and called the following day; no adverse effects were observed or reported.

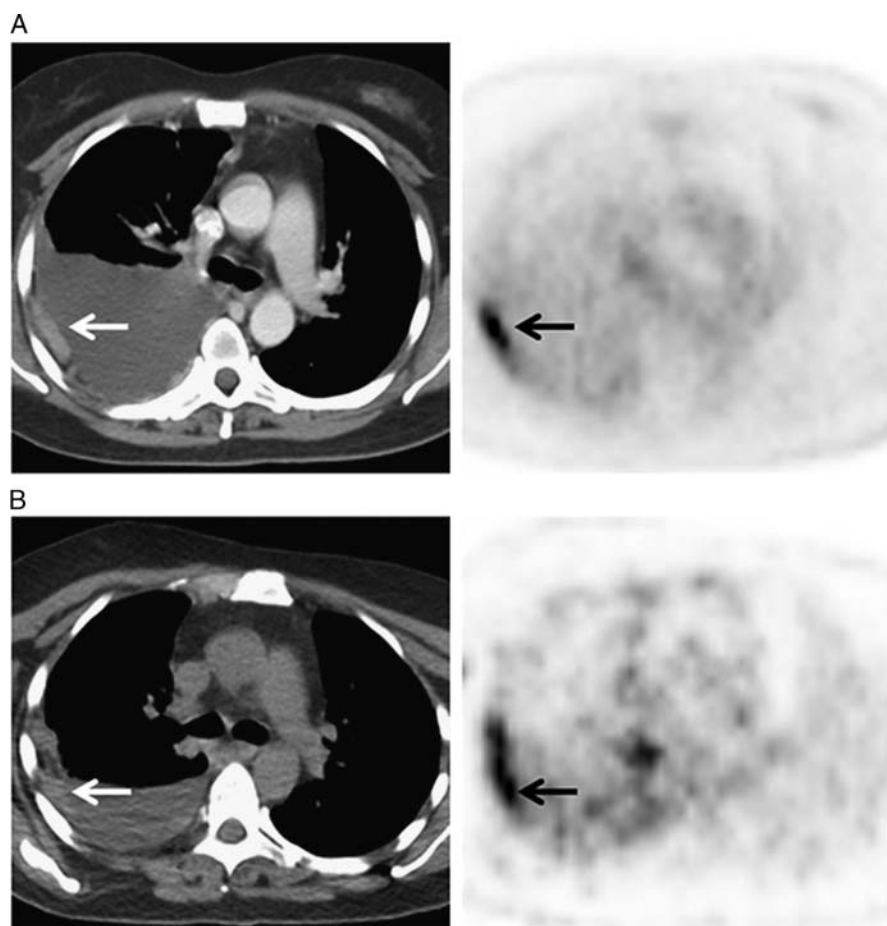


FIGURE 1. A 47-year-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma and known metastases in the liver, nodes, and pleura. **A**, Axial CT and PET images from a contrast-enhanced FDG PET/CT through the chest demonstrate FDG-avid right pleural masses (SUVmax, 6.0; arrows). **B**, Axial CT and PET images from a non-contrast-enhanced ^{89}Zr -trastuzumab PET/CT at the same level demonstrate ^{89}Zr -trastuzumab avidity in the pleural lesions (SUVmax, 6.9; arrows).

Vital signs were recorded before and after injection, and no changes of clinical significance were observed.

In 4 (36%) of 11 patients, both readers observed foci of ⁸⁹Zr-trastuzumab avidity considered suggestive of HER2-positive disease. In 2 patients, the suspicious ⁸⁹Zr-trastuzumab foci were in pleura, whereas 1 patient exhibited suspicious ⁸⁹Zr-trastuzumab foci in the bone, and 1 patient exhibited suspicious ⁸⁹Zr-trastuzumab foci in the liver.

Biopsy and Pathologic Evaluation of Suspicious ⁸⁹Zr-Trastuzumab Foci

Image-guided biopsy was performed in all 4 patients with suspicious ⁸⁹Zr-trastuzumab-avid foci. Patient 11 was a 40-year-old woman who was diagnosed in October 2013 as having an estrogen receptor (ER)-positive/HER2-negative primary invasive ductal carcinoma. At the time of protocol enrollment, an FDG PET/CT demonstrated FDG-avid bone, liver, nodal, and chest wall malignancy. Bone and chest wall lesions had been pathologically confirmed as metastases. ⁸⁹Zr-trastuzumab PET/CT was performed in March 2016 and demonstrated ⁸⁹Zr-trastuzumab-avid osseous and nodal lesions. The most avid lesion was a right femoral focus (SUVmax, 10.1). Biopsy of the right femoral focus demonstrated HER2 IHC of 2+ and FISH of 1.6. As FISH was less than 2.0, this was considered a false-positive ⁸⁹Zr-trastuzumab focus.

Patient 14 was a 47-year-old woman diagnosed in June 2011 as having an ER-positive/HER2-negative primary invasive ductal

carcinoma. At the time of protocol enrollment, an FDG PET/CT demonstrated FDG-avid liver, nodal, and pleural malignancy (Fig. 1). Hepatic and pleural lesions had been pathologically confirmed as metastases. ⁸⁹Zr-trastuzumab PET/CT was performed in May 2016 and demonstrated ⁸⁹Zr-trastuzumab-avid pleural (SUV, 6.9) and liver (SUV, 10.9) lesions. The pleural lesion was selected for biopsy as the lesion with the lowest risk for sampling. Biopsy of the pleural lesion demonstrated HER2 IHC of 2+ and FISH of 2.4. As FISH was greater than 2.0, this was considered a true-positive ⁸⁹Zr-trastuzumab focus for a HER2-positive distant metastasis.

Patient 17 was a 51-year-old woman diagnosed in November 2010 as having an ER-positive/HER2-negative primary invasive ductal carcinoma. At the time of protocol enrollment, an FDG PET/CT demonstrated FDG-avid bone, nodal, and lung malignancy (Fig. 2). A lung lesion had been pathologically confirmed as metastasis. ⁸⁹Zr-trastuzumab PET/CT was performed in May 2016 and demonstrated ⁸⁹Zr-trastuzumab-avid lung (SUV, 11.3) lesions. Biopsy of a lung lesion demonstrated HER2 IHC of 1+, which was considered a false-positive ⁸⁹Zr-trastuzumab focus.

Patient 20 was a 58-year-old woman diagnosed in August 2003 as having an ER-positive/HER2-negative primary mixed invasive ductal and lobular carcinoma. At the time of protocol enrollment, an FDG PET/CT demonstrated FDG-avid bone, liver, and nodal malignancy (Fig. 3). Bone and liver lesions had been pathologically confirmed as metastases. ⁸⁹Zr-trastuzumab PET/CT was performed in June 2016 and demonstrated ⁸⁹Zr-trastuzumab-avid liver (SUV, 15.7) lesions. Biopsy of a liver lesion demonstrated

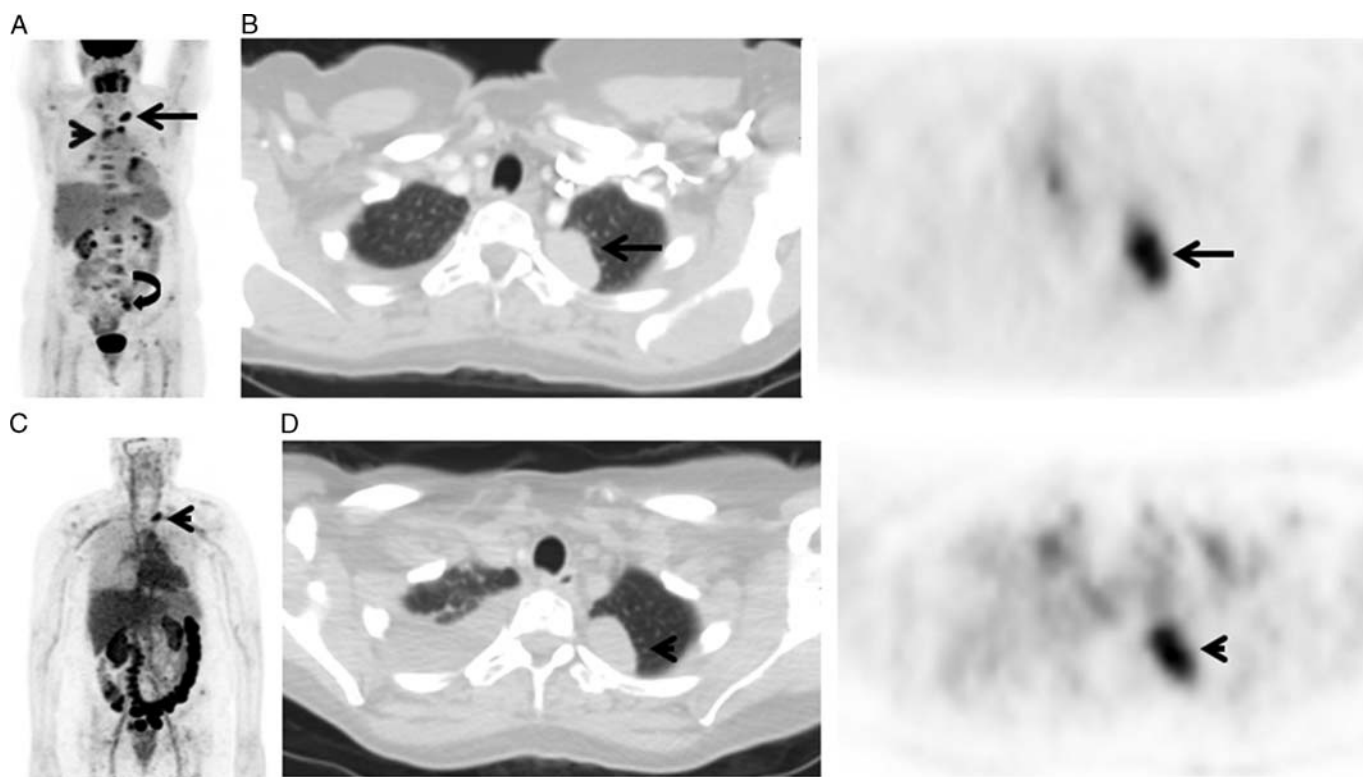


FIGURE 2. A 51-year-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma. **A**, FDG PET MIP demonstrates multiple FDG-avid metastases, which correspond to lesions in the lung (arrow), nodes (arrowhead), and bone (curved arrow). **B**, Axial CT and PET images from a contrast-enhanced FDG PET/CT through the chest, displayed in lung CT windows, demonstrate an FDG-avid left lung mass (SUVmax, 7.3; arrow). **C**, ⁸⁹Zr-trastuzumab PET MIP demonstrates physiologic tracer in the blood pool, liver, and bowel, as well as a suspicious ⁸⁹Zr-trastuzumab-avid focus in the upper chest (arrow). **D**, Axial CT and PET images from a non-contrast-enhanced ⁸⁹Zr-trastuzumab PET/CT demonstrate that the suspicious ⁸⁹Zr-trastuzumab-avid focus corresponds to the left lung mass (SUVmax, 11.3; arrowhead).

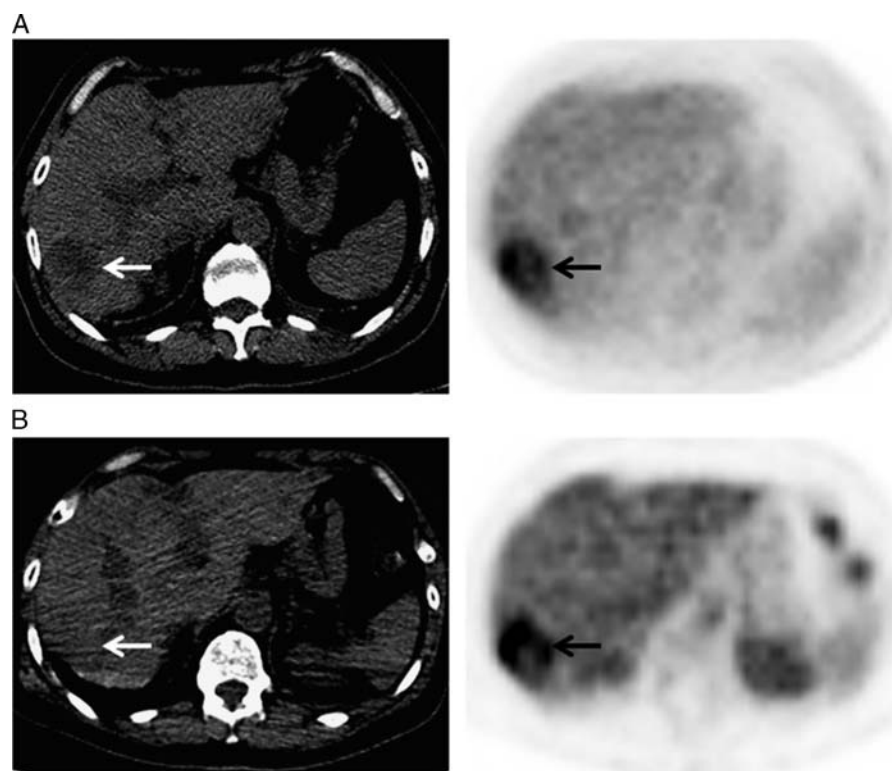


FIGURE 3. A 58-year-old woman with primary ER-positive, HER2-negative invasive ductal breast carcinoma and known metastases in the bone, liver, and nodes. **A**, Axial CT and PET images from a non-contrast-enhanced FDG PET/CT through the abdomen demonstrate an FDG-avid liver metastasis (SUVmax, 5.6; arrows). **B**, Axial CT and PET images from a non-contrast-enhanced ⁸⁹Zr-trastuzumab PET/CT at the same level demonstrate ⁸⁹Zr-trastuzumab avidity in the liver metastasis (SUVmax, 15.7; arrows).

HER2 IHC of 1+, which was considered a false-positive ⁸⁹Zr-trastuzumab focus. The remaining 7 patients did not have suspicious foci of ⁸⁹Zr-trastuzumab avidity.

DISCUSSION

This study describes a second group of 11 patients in a prospective clinical trial of HER2-targeted imaging with ⁸⁹Zr-trastuzumab PET/CT to evaluate for unsuspected HER2-positive metastases in patients with HER2-negative primary breast cancer. It demonstrates that in a small subset of patients with HER2-negative primary breast cancer HER2-targeted imaging can detect previously unsuspected HER2-positive disease.

Between the current group of 11 patients and a previously published initial group of 9 patients,⁷ a completed cohort of 20 patients with HER2-negative primary breast cancer have now been evaluated. In total, 3 (15%) of 20 patients were demonstrated to have biopsy-proven unsuspected HER2-positive metastases. In another 6 (30%) of 20 patients, suspicious ⁸⁹Zr-trastuzumab-avid foci resulted in biopsies with HER2-negative pathology, which are being conservatively considered false-positive ⁸⁹Zr-trastuzumab-avid foci.

While medical imaging of oncology patients has traditionally been used for the detection of tumors, staging, and evaluation of treatment response, this study suggests that targeted medical imaging may be able to help select patients for targeted systemic therapies. More than 900,000 women are living with metastatic breast cancer, with more than 50,000 diagnosed each year.¹⁰ Eighty percent of these women have HER2-negative primary malignancies.

If 15% of patients with metastatic HER2-negative primary breast cancer could be found to harbor HER2-positive metastases, this would represent a current population of 135,000 women. Thus, targeted HER2 imaging has the potential to play a clinically valuable role in identifying patients who may benefit from HER2-targeted therapies.

A limitation of this approach is the currently high level of presumably false-positive suspicious ⁸⁹Zr-trastuzumab-avid foci. This leads to unneeded workup and biopsies. The reason for these false-positive foci is unclear. One possibility is pathologic sampling or processing errors. For example, decalcification of osseous lesions may decrease the intensity of HER2 IHC staining, resulting in a false-negative pathologic result.¹¹ If a lesion exhibits heterogeneity of HER2 expression, it is possible that in any one biopsy the HER2-positive malignancy was undersampled. Although this could result in erroneous pathologic analysis, given the high number of false-positives, it is likely that at least some represent nonspecific ⁸⁹Zr-trastuzumab uptake in HER2-negative lesions. Another possible explanation is free ⁸⁹Zr being released from its chelator during the long 5-day uptake period. When loosely chelated, ⁸⁹Zr is known to be a bone seeker.¹² Indeed, evidence suggests that when ⁸⁹Zr is chelated to antibodies with desferrioxamine radioactivity accumulates in the bone.¹³ Thus, nonspecific binding of potentially free ⁸⁹Zr at sites of osseous turnover associated with bone metastases could result in false-positive osseous foci. Another possible explanation for presumed false-positive ⁸⁹Zr-trastuzumab-avid foci could be related to in vivo HER2 internalization rates or affinity differences that would not necessarily be reflected by IHC or FISH.¹⁴ More specific HER2-targeting radiotracers will need to

be explored. One potential alternative is ⁸⁹Zr-pertuzumab, which demonstrated HER2 specificity in animal models.¹⁵

Human epidermal growth factor receptor 2–negative patients have been reported to respond to trastuzumab therapy. It would be of interest to evaluate if patients with positive ⁸⁹Zr-trastuzumab scans but negative HER2 status by conventional criteria respond to trastuzumab. This would raise the potential of using ⁸⁹Zr-trastuzumab as a predictive biomarker¹⁶ and selecting patients for HER2-target therapy based on imaging.

It is interesting to note that the intensity of ⁸⁹Zr-trastuzumab avidity did not discriminate between true-positive and false-positive lesions for HER2 expression. The most ⁸⁹Zr-trastuzumab-avid lesion in the study was a hepatic lesion (patient 20; SUV, 15.7), which had a HER2-negative pathology. Likewise, a substantially less ⁸⁹Zr-trastuzumab-avid lesion (patient 14; SUV, 6.9) had HER2-positive pathology and was a true-positive.

Another interesting issue was that even when a ⁸⁹Zr-trastuzumab-avid focus was considered a false-positive, there could be noticeable heterogeneity in HER2 expression between the primary malignancy and the avid metastasis. For example, patient 11 had a primary malignancy with an IHC of 0. The ⁸⁹Zr-trastuzumab-avid osseous metastasis that was biopsied had an IHC of 2+ and FISH of 1.6. While IHC 2+/FISH 1.6 is considered HER2-negative by ASCO guidelines, it still represents a change from the primary malignancy. The value of ⁸⁹Zr-trastuzumab PET/CT for evaluating this type of heterogeneity needs further evaluation.

The strength of this study is its design as a prospective clinical trial, whereas its weakness is still the small sample size. Ethical standards and logistical obstacles prevent biopsy of all ⁸⁹Zr-trastuzumab foci; thus, pathology is classified on the basis of a limited number of biopsies. Likewise, ethical standards prevent biopsy confirmation of HER2-negative status of metastases in patients without suspicious foci on ⁸⁹Zr-trastuzumab imaging.

In conclusion, ⁸⁹Zr-trastuzumab PET/CT can be used to detect unsuspected HER2-positive metastases in a subset of patients with HER2-negative primary breast cancer. This demonstrates that targeted imaging can identify patients amenable to targeted therapies. Presumed false-positive results currently limit the translation of these techniques, but there is great potential for more specific methodology to have substantial clinical value in the future.

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First-in-human HER2-targeted imaging using ^{89}Zr -pertuzumab PET/CT: Dosimetry and clinical application in patients with breast cancer

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ABSTRACT

In this first-in-human study, we evaluate the safety and dosimetry of ^{89}Zr -pertuzumab PET/CT (Positron Emission Tomography / Emission Tomography) for HER2 (Human epidermal growth factor receptor 2)-targeted imaging in patients with HER2-positive breast cancer.

Materials and Methods: Patients with HER2-positive breast cancer and evidence of distant metastases were enrolled in an Institutional Review Board-approved prospective clinical trial. Pertuzumab was conjugated with deferoxamine and radiolabeled with ^{89}Zr . Patients underwent ^{89}Zr -pertuzumab PET/CT with 74 MBq of ^{89}Zr -pertuzumab in a total antibody mass of 20-50 mg of pertuzumab. PET/CT, whole-body probe counts, and blood draws were performed over 8 days to assess pharmacokinetics, biodistribution, and dosimetry. PET/CT images were evaluated for ability to visualize HER2-positive metastases.

Results: Six patients with HER2-positive metastatic breast cancer were enrolled and administered ^{89}Zr -pertuzumab. No toxicities occurred. Dosimetry estimates from Organ Level Internal Dose Assessment (OLINDA) demonstrated the organs receiving the highest doses (mGy/MBq) were liver (1.75 ± 0.21), kidneys (1.27 ± 0.28), and heart wall (1.22 ± 0.16) with an average effective dose of 0.54 ± 0.07 mSv/MBq. PET/CT demonstrated optimal imaging 5-8 days post-administration. ^{89}Zr -pertuzumab was able to image multiple sites of malignancy, and suggest they are HER2-positive. In two patients with both known HER2-positive and HER2-negative primary breast cancers and brain metastases, ^{89}Zr -pertuzumab PET/CT suggested the brain metastases were HER2-positive. In one of two patients, subsequent resection of a brain metastasis proved HER2-positive disease, confirming ^{89}Zr -pertuzumab-avidity was true positive for HER2-positive malignancy.

Conclusion: This first-in-human study demonstrated safety, dosimetry, biodistribution, and successful HER2-targeted imaging with ^{89}Zr -pertuzumab PET/CT. Potential clinical applications

include assessment of HER2 status of lesions which may not be accessible to biopsy and assessment of HER2 heterogeneity.

INTRODUCTION

Human epidermal growth factor receptor 2 (HER2) is a critical biomarker in breast cancer, and its expression directly influences treatment. Approximately 20% of invasive ductal breast malignancies are classified as HER2-positive as a result of *ERBB2* gene amplification and/or the subsequent overexpression of the HER2 protein on the surface of tumor cells (1). Patients with HER2-positive breast cancer receive specific therapies targeted to HER2 that reduce the risk of death, while patients with HER2-negative breast cancer do not receive them (2,3). This has resulted in considerable interest in HER2-targeted imaging (4). Recent work has demonstrated the ability to detect HER2-positive metastases in patients with HER2-negative primary breast tumors both by HER2-targeted imaging confirmed with immunohistochemistry (5,6) and molecular analyses (7). Thus, the ability to perform non-invasive, whole body, targeted HER2 imaging may be valuable in the detection of otherwise unsuspected HER2-positive malignancy and may help direct patients to appropriate HER2-targeted therapy.

While there have been successes in HER2-targeted imaging with ^{89}Zr -trastuzumab, there have also been examples of non-specific visualization of malignancy that is HER2-negative on pathology (5,6). More specific HER2-targeted agents may be needed for clinical translation of HER2-targeted imaging agents. Pertuzumab is a newer humanized monoclonal antibody that binds to the HER2 receptor at a site distinct from trastuzumab and appears to be more efficient than trastuzumab (8). *In vitro* and *in vivo* models have demonstrated successful ^{89}Zr -pertuzumab targeting to HER2-positive malignancy, and have notably demonstrated increased affinity for HER2 in the presence of trastuzumab (9), as may be the case in patients with HER2-positive malignancies receiving trastuzumab. Here we present our results from first-in-human HER2-

targeted imaging with ^{89}Zr -pertuzumab PET/CT, in order to document safety, dosimetry, and potential clinical utility of this HER2-targeted imaging agent.

MATERIALS AND METHODS

Patients

This study was performed under a single-center prospective Memorial Sloan Kettering Cancer Center Institutional Review Board-approved protocol (ClinicalTrials.gov identifier NCT03109977). All patients provided written informed consent. Patients with pathologically proven HER2-positive metastatic breast cancer were identified as potential candidates. HER2 positivity was defined according to American Society of Clinical Oncology guidelines (10), including 3+ HER2 immunohistochemistry (IHC) or 2+ HER2 IHC with ≥ 2.0 HER2 amplification on fluorescence in situ hybridization (FISH); 0 or 1+ IHC or 2+ IHC with < 2.0 HER2 amplification on FISH was considered HER2-negative. Inclusion criteria were: 1) biopsy-proven, HER2-positive malignancy, 2) foci of malignancy on imaging within 60 days of enrollment, 3) women age > 21 , and 4) Eastern Cooperative Oncology Group performance score of 0-2. Exclusion criteria were: 1) life expectancy < 3 months, 2) pregnancy or lactation, and 3) inability to undergo PET/CT scanning because of weight limits. Biopsies demonstrating HER2-positive malignancy were required for inclusion. The HER2-positive biopsy was allowed at any time of the patient's disease course, and was allowed from the primary breast malignancy or a site of metastatic disease. Patients were allowed to be on HER2 directed therapy. Sites of known malignancy were determined by medical imaging, including CT, MR, and ^{18}F -Fluorodeoxyglucose (^{18}F -FDG) PET/CT, within 60 days of protocol enrollment. The primary

malignancy and at least one site of distant metastasis were pathological proven as part of inclusion criteria.

⁸⁹Zr-pertuzumab

The ⁸⁹Zr-DFO-pertuzumab was manufactured at the MSK Radiochemistry and Molecular Imaging Probes Core Facility in compliance with the requirements specified in the Chemistry, Manufacturing, and Controls section of an United States Food and Drug Administration-acknowledged Investigational New Drug application (#134411). The preparation process involved conjugating clinical-grade pertuzumab (Perjeta, Genentech, South San Francisco, CA) with a bifunctional chelator, p-SCN-Bn-Deferoxamine (Macrocyclics, Plano, TX), followed by radiolabeling with ⁸⁹Zr, a radiometal positron emitter with a 78.4-hour radioactive half-life. The conjugation and radiolabeling were performed using methodology previously described (11). Radiolabeling with ⁸⁹Zr was chosen based on this metallo-radionuclide's favorable properties such as radioactive half-life, which is long enough to allow for imaging of radiolabeled antibodies after localization at the target site has occurred, as well as mild radiolabeling conditions (at ambient temperature in 1M ammonium acetate buffer, pH7), which help to preserve pertuzumab protein integrity and immunoreactivity during the radiolabeling process (12,13). The ⁸⁹Zr-DFO pertuzumab final drug product batches underwent quality control testing prior to batch release for patient administration, in order to ensure conformance to the following acceptance specifications: radiochemical purity as determined by radio thin layer chromatography and size exclusion high-performance liquid chromatography; radio-immunoreactivity, as determined by using a live antigen expressing cell binding assay; endotoxin content, as measured by the portable test system supplied by Charles River Laboratories

(Wilmington, Massachusetts, USA); sterilizing filter integrity, as measured by the bubble point method; pH as measured by pH strips; appearance as a clear and particle free-solution, as determined by visual inspection check; and radionuclidic identity verification, as measured by radioactive gamma spectroscopy. Sterility testing, using the direct media inoculation method, was performed post-release.

⁸⁹Zr-pertuzumab Administration

An intravenous line was established and flushed with 5% human serum albumin solution. An amount of 18 or 48 mg of non-radiolabeled pertuzumab was then intravenously administered over 5 minutes. Cold pertuzumab was administered to help reduce non-specific uptake of the subsequent radiolabeled pertuzumab. Then 74 MBq +/- 10% of ⁸⁹Zr-pertuzumab was intravenously administered in a mass of approximately 2 mg, to bring the total pertuzumab antibody mass to 20 or 50 mg for each patient. The first two patients were administered 50 mg, then antibody mass was reduced to 20 mg for the next two patients. Visual analysis suggested 50 mg total antibody mass produced lower background uptake, thus the total antibody mass was increased back to 50 mg for the final two patients. Patients were monitored for side effects on the day of and the day after ⁸⁹Zr-pertuzumab administration.

⁸⁹Zr-pertuzumab PET/CT and Image Analysis

Up to four whole body PET/CT scans were obtained for each patient on days 1, 2-4, 5-6, and 7-8 following administration (day 0) of ⁸⁹Zr-pertuzumab. Ranges for days of imaging were preselected before opening the protocol to allow both comprehensive multiday imaging and flexibility of scheduling, particularly over the weekend.

Patients were imaged from skull apex to mid-thigh on a dedicated research PET/CT scanner (GE Discovery 710) in 3D mode with emission time per bed position extending from 4 min (day 1) to 8 min (day 7-8). Low dose CT scans were acquired with an x-ray tube current of 80 mA. PET/CT images were reconstructed with attenuation, scatter, and other standard corrections applied and using iterative reconstruction. ^{89}Zr -pertuzumab PET/CT scans were interpreted by a nuclear radiologist with experience in HER2-targeted imaging (GAU) and knowledge of the patient's medical history and prior imaging. Non-physiologic radiotracer uptake was considered suspicious for HER2-positive malignancy. Volumes of interest were drawn on PET/CT images over normal liver, kidney, spleen, and lung using a dedicated workstation (Hermes Medical Solution, Stockholm, Sweden). Normal tissue uptake was quantified by mean standardized uptake value adjusted to lean body mass (SUV_{LBM}).

Whole-body and Serum Clearance Measurements

Whole-body clearance was determined by serial measurements of count-rate using a 12.7 cm-thick sodium iodide NaI (Tl) scintillation detector at a fixed 3 m from the patient. Background-corrected geometric mean counts were obtained after infusion before and after first voiding and subsequently at the times of the PET scans ($n=6$). Count rates were normalized to the immediate post-infusion value (taken as 100%) to yield relative retained activities (in %). Multiple blood samples were obtained at approximately 15 min, 30 min, and 1-2 h after injection, and subsequently at the times of each PET scan ($n=7$). Aliquots of serum were counted using a gamma well-type detector (Wallac Wizard 1480 gamma counter, Perkin Elmer) and measured activity concentrations converted to percent injected activity/liter (% IA/L).

A mono-exponential function was fitted to the whole-body probe data and a bi-exponential function fitted to the serum activity concentration data using SAAM software (14). Areas under the curve (AUC) and corresponding residence times were derived by analytic integration.

Normal Tissue Dosimetry

Normal tissue dose estimates were derived as described previously (15,16). Briefly, image-derived SUV_{LBM} were converted to activity concentration per unit mass (kBq/g) and AUC estimated by trapezoidal integration. Whole-organ AUCs were estimated by multiplying the activity concentration AUC by projected organ mass. Residence times were derived by dividing whole organ AUC by the administered activity. Corresponding values for heart contents and red marrow were estimated from the serum AUC (17). The residence time for the remainder of body was derived by subtracting all individually estimated residence times from the WB residence time. Thereafter, absorbed radiation doses to individual organs were calculated using the OLINDA/EXM software application (18).

Comparison of ^{89}Zr -pertuzumab Dosimetry with ^{89}Zr -trastuzumab Dosimetry

Tissue and total body dosimetry was compared for the newly calculated values for ^{89}Zr -pertuzumab and published values for ^{89}Zr -trastuzumab.

Statistics

Kinetic parameters and absorbed dose estimates were calculated for each patient on an individual basis. Subsequently, these were summarized using descriptive statistics.

RESULTS

Patient Characteristics

Between April and June 2017, six patients, all women with biopsy-proven HER2-positive malignancy from invasive ductal breast cancer (IDC), completed the study protocol. All patients underwent imaging on days 1, 2-4, 5-6, and/or 7-8, as prescribed prospectively in the protocol. Patient characteristics are summarized in Table 1.

Sites of Known Malignancy at Time of Protocol Enrollment

Sites of known malignancy were determined from medical imaging within 60 days of protocol enrollment. Known nodal disease was present in four patients, brain malignancy in two, hepatic malignancy in two, and malignancy involving the breast, bone, chest wall, and lung each in one patient. Sites of known malignancy are summarized in Table 1.

Adverse Events

All six patients underwent ^{89}Zr -pertuzumab administration. Patients were monitored for two hours after tracer injection, as well as evaluated the following day when they returned for PET/CT imaging, and no side effects were observed or reported. Vital signs were recorded before and after tracer administration and there were no changes with clinical significance. Safety data were reviewed and approved by the US Food and Drug Administration as part of an Investigational New Drug application.

Pharmacokinetics

Whole body and serum clearance conformed to mono- and bi-exponential kinetics, respectively. Summed biologic clearance curves are shown in Figure 1. Summary statistics for the clearance parameters are provided in Table 2.

Biodistribution and Normal Tissue Dose Estimates

^{89}Zr -pertuzumab uptake was observed in the blood pool, liver, kidney, and spleen. There was little measureable change in whole body activity following the first void ($98\% \pm 1.8$ of pre-void measurement). The urinary bladder was not visualized on any PET/CT scan. Bowel excretion was visualized in two patients on day 1 and 2 scans. Uptake in liver and kidneys in terms of SUV_{LBM} was relatively constant over the duration of imaging, whereas blood pool and spleen uptake decreased over time. These sites of tracer visualization were considered physiologic. Absorbed dose estimates for normal tissues are provided in Table 3. The organs receiving the highest doses (mGy/MBq) were liver (1.75 ± 0.21), kidneys (1.27 ± 0.28), and heart wall (1.22 ± 0.16), with an average effective dose of $0.54 \pm 0.07 \text{ mSv}/\text{MBq}$.

Comparison of ^{89}Zr -pertuzumab Dosimetry with ^{89}Zr -trastuzumab Dosimetry

The mean effective dose of ^{89}Zr -pertuzumab was $0.54 \text{ mSv}/\text{MBq}$. Figure 2 shows the comparative absorbed dose estimates for both antibodies.

Imaging of Lesions with ^{89}Zr -pertuzumab PET/CT

Patient 1 had two known primary breast malignancies, a right breast estrogen receptor (ER), progesterone receptor (PR), and HER2-positive (HER2 IHC 3+) IDC diagnosed in 2014 and the other ER, PR, and HER2-negative (HER2 IHC 0) IDC diagnosed in 2015. She had

received HER2 directed therapy, including TDM-1 in 2015 and was currently on trastuzumab therapy at the time of the ^{89}Zr -pertuzumab PET/CT. She had a recent diagnosis of brain metastases. ^{89}Zr -pertuzumab PET/CT demonstrated progressive increase in ^{89}Zr -pertuzumab avidity over PET/CT scans obtained on days 1, 2, 6, and 8 following tracer administration (SUVmax of the most avid lesion was 13.6, 16.6, 26.0, and 30.1 on these four days; see Fig. 3A-D). Blood pool activity, including activity in the superior sagittal sinus, demonstrated continued decrease over the scans. This allowed most optimal visualization of the known brain metastases (Fig. 3E) on the day 8 scan (Fig. 3F).

Patient 2 had a left breast ER, PR, and HER2+ (HER2 IHC 2+, HER2 amplification 4.0 on FISH) IDC diagnosed in 2014. She had received HER2 directed therapy, including TDM-1 in 2015-2016 and was currently on trastuzumab and pertuzumab therapy at the time of the ^{89}Zr -pertuzumab PET/CT. She had known supraclavicular nodal metastases at the time of ^{89}Zr -pertuzumab PET/CT. There was relatively stable ^{89}Zr -pertuzumab avidity on days 1, 2, 5, and 7 following tracer administration (SUVmax of the most avid lesion was 6.0, 4.7, 4.6, and 5.1 on these four days).

Patient 3 had a left breast ER, PR, and HER3+ (HER2 IHC 3+) IDC diagnosed in 2014, which was metastatic to the lung at the time of diagnosis. She had received HER2 directed therapy, including trastuzumab and pertuzumab, which she was receiving at the time of the ^{89}Zr -pertuzumab PET/CT. At the time of ^{89}Zr -pertuzumab PET/CT, she had demonstrable ^{18}F -FDG-avid disease in the left breast and left axillary nodes. There was mildly increasing ^{89}Zr -pertuzumab avidity on days 1, 2, 6, and 7 following tracer administration (SUVmax of the breast

lesion was 3.7, 4.7, 6.3, and 5.5). Decreasing background avidity made the avid lesions best visible on the day 6 and 7 scans (Fig. 4). ^{89}Zr -pertuzumab avidity was similar to ^{18}F -FDG avidity (SUVmax 6.6), which was performed 3 weeks prior.

Patient 4 was diagnosed with ER-positive, HER2-negative (HER2 IHC 1+) in 2003. At the time of ^{89}Zr -pertuzumab PET/CT, she had demonstrable disease in the liver, bone, and chest wall on CT and MR. She had previously received HER2 directed therapy, including trastuzumab and pertuzumab, which she was receiving at the time of the ^{89}Zr -pertuzumab PET/CT. A recent biopsy of a right chest wall mass was HER2-positive (HER2 IHC 2+, HER2 amplification 2.4 on FISH). There was low-level avidity in the right chest wall lesion (SUVmax 2.8, 2.3, 2.4, and 2.4 on days 1, 4, 5, and 8 post- ^{89}Zr -pertuzumab administration).

Patient 5 was diagnosed with two distinct right breast malignancies in 2014 and an ER-negative, HER2-positive (HER2 IHC 3+) malignancy, as well as an ER-positive, HER2-negative (HER2 IHC 0) malignancy. She had previously received HER2 directed therapy, including trastuzumab and pertuzumab, which she was receiving at the time of the ^{89}Zr -pertuzumab PET/CT. At the time of ^{89}Zr -pertuzumab PET/CT, she had demonstrable disease in the brain, lung, nodes, and liver. There was mild ^{89}Zr -pertuzumab avidity in the brain, lung, and nodal lesions, and was greatest in the brain (SUVmax of the most avid brain lesion was 2.9, 6.1, and 6.1 on days 1, 2, and 5 post-tracer administration). A brain metastasis was resected and was HER2-positive (IHC 2+, HER2 amplification 2.4 on FISH), confirming this was a site ^{89}Zr -pertuzumab-avidity which was true positive for HER2-positive malignancy. Liver lesions were not appreciably ^{89}Zr -pertuzumab-avid above liver background.

Patient 6 had a right breast ER-negative, HER2-positive (HER2 IHC 3+) IDC diagnosed in 2008. She had previously received HER2 directed therapy, including trastuzumab and pertuzumab, and was currently on TDM-1 therapy at the time of the ^{89}Zr -pertuzumab PET/CT. At the time of ^{89}Zr -pertuzumab PET/CT, she had only small volume disease in thoracic and abdominal nodes. Avidity in small nodes, greatest 1.5 x 1.2 cm in the right common iliac chain, was difficult to appreciate on ^{89}Zr -pertuzumab PET/CT.

DISCUSSION

This first-in-human trial demonstrates safety and dosimetry for intravenously administered ^{89}Zr -pertuzumab, which has been reviewed and accepted by the US Food and Drug Administration. We have also demonstrated successful HER2-targeted imaging in patients with HER2-positive metastatic breast cancer.

The mean effective dose of ^{89}Zr -pertuzumab was 0.54 mSv/MBq, which is comparable with other radiolabeled antibody PET tracers such as ^{89}Zr -J591 (0.38 mSv/MBq) (15). In particular, the biodistribution and normal tissue dosimetry for ^{89}Zr -pertuzumab is comparable with ^{89}Zr -trastuzumab (0.48 mSv/MBq) (16). However, ^{89}Zr -pertuzumab does appear to have slightly higher uptake in the central parenchymal organs (liver, kidney, spleen, and lung) than ^{89}Zr -trastuzumab; this translates into a higher (mGy/MBq) dose by an average factor of approximately 1.3 for these organs. Due to their relatively slow kinetics, antibody-based PET tracers require radionuclides with relatively long physical half-lives. This leads to higher

radiation doses than small molecule imaging agents that have fast kinetics and can use radionuclides with relatively short physical half-times — the classic example of which is ^{18}F -FDG. In this study, we used a lower activity of ^{89}Zr (~74 MBq) than what we have used in our previous studies (~185 MBq). This was found to be adequate in terms of image quality for clinically feasible emission times, ranging from 4-8 minutes per bed position. As radiation dose is directly proportional to administered activity, this represents a significant reduction in actual (mGy) dose compared to our previous studies. It is also of note that several investigators including equipment manufacturers are developing new advanced methods of image reconstruction that should result in further significant reductions in radiation dose. The radiation exposures generated by radiolabeled antibody PET tracers would be justified if they produce clinically valuable information.

Imaging of ^{89}Zr -pertuzumab was best performed 5-8 days following tracer administration. Scans on earlier days had higher liver and blood pool backgrounds and tended to have lower tumor uptake. The combination of higher tumor uptake and lower background on 5-8 day imaging has been observed with other antibody tracers (*15,16*). The multiple-day delay in blood pool clearance of antibody tracers can be considered a limitation of this technology. Potential alternatives include affibodies (*19*) and nanobodies (*20,21*), which have rapid biodistribution, allowing for imaging within hours of tracer administration. Affibodies and nanobodies labelled with shorter half-life tracers may have the added advantage of lower radiation dose to patients.

Although limited activity was seen in the bowel, we anticipate that this was the primary route for ^{89}Zr -pertuzumab excretion that did occur. Little measureable change was observed in

whole body activity following the first void ($98\% \pm 1.8$ of pre-void measurement) and there was no visualization of activity in the urinary bladder at any time.

One potential clinical application of ^{89}Zr -pertuzumab PET/CT is the assessment of HER2 status of disease, which may not be accessible by biopsy; for example, brain metastases. This becomes increasingly important given the recent finding that in patients with HER2-negative primary breast cancer who develop brain metastases, 20% will acquire HER2-positive metastases (7). Thus, a method of non-invasive, whole body screening for HER2-positive disease would be of clinical value as a predictive biomarker (22), helping to select patients for HER2-targeted therapy based on imaging. In this small trial, patients 1 and 5 had both HER2-positive and HER2-negative primary breast cancers and brain metastases. ^{89}Zr -pertuzumab PET/CT imaging suggested that the brain metastases were HER2-positive, one with histologic proof.

Another potential clinical application of ^{89}Zr -pertuzumab PET/CT is the assessment of HER2 heterogeneity. Patient 4 had a biopsy-proven HER2-negative primary breast malignancy, but a biopsy-proven HER2-positive chest wall metastases. Two previous liver biopsies in this patient were HER2-negative. The only site of ^{89}Zr -pertuzumab avidity was the chest wall lesion, which was the only site of known HER2-positive disease as defined by American Society of Clinical Oncology criteria. Of course, not all sites of malignancy had histologic analysis, but this early work suggests that ^{89}Zr -pertuzumab could assess heterogeneous HER2 tumor burden.

This first-in-human pilot of ^{89}Zr -pertuzumab PET/CT has a sample size of only six patients and was primarily designed to provide normal tissue biodistribution and dosimetry data. Further information on the biodistribution of ^{89}Zr -pertuzumab in normal and malignant tissues will be generated in additional HER2-targeted imaging trials with this novel agent. Patients included in this trial had metastases that were often treated with systemic therapy, often

including HER2-targeted therapy, prior to trial enrollment. This introduces difficulties in comparing extent of active malignancy with radiotracer uptake. A treated HER2-positive metastasis may not have sufficient residual active tumor to visualize on PET. Despite this limitation, five of six patients demonstrated sites of disease that were avid for ^{89}Zr -pertuzumab. Patients who had previously received, and were currently receiving, HER2-targeted therapy still demonstrated ^{89}Zr -pertuzumab-avid lesions. The effects of HER2-targeted therapy on ^{89}Zr -pertuzumab are not known; however, it was clear that HER2-targeted therapy did not prevent HER2-targeted imaging with ^{89}Zr -pertuzumab.

CONCLUSION

This first-in-human trial demonstrates that ^{89}Zr -pertuzumab PET/CT may be safely performed and has the potential to be a clinically valuable HER2-targeted imaging agent for patients with metastatic breast cancer. Potential clinical applications include assessment of HER2 status of lesions which may not be accessible to biopsy and assessment of HER2 heterogeneity. ^{89}Zr -pertuzumab PET/CT will next be utilized in a prospective clinical trial of patients with HER2-negative primary breast cancer, in order to analyze the ability of ^{89}Zr -pertuzumab to detect unsuspected HER2-positive metastatic disease and help direct HER2 targeted therapy to appropriate patients.

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FIGURE LEGENDS

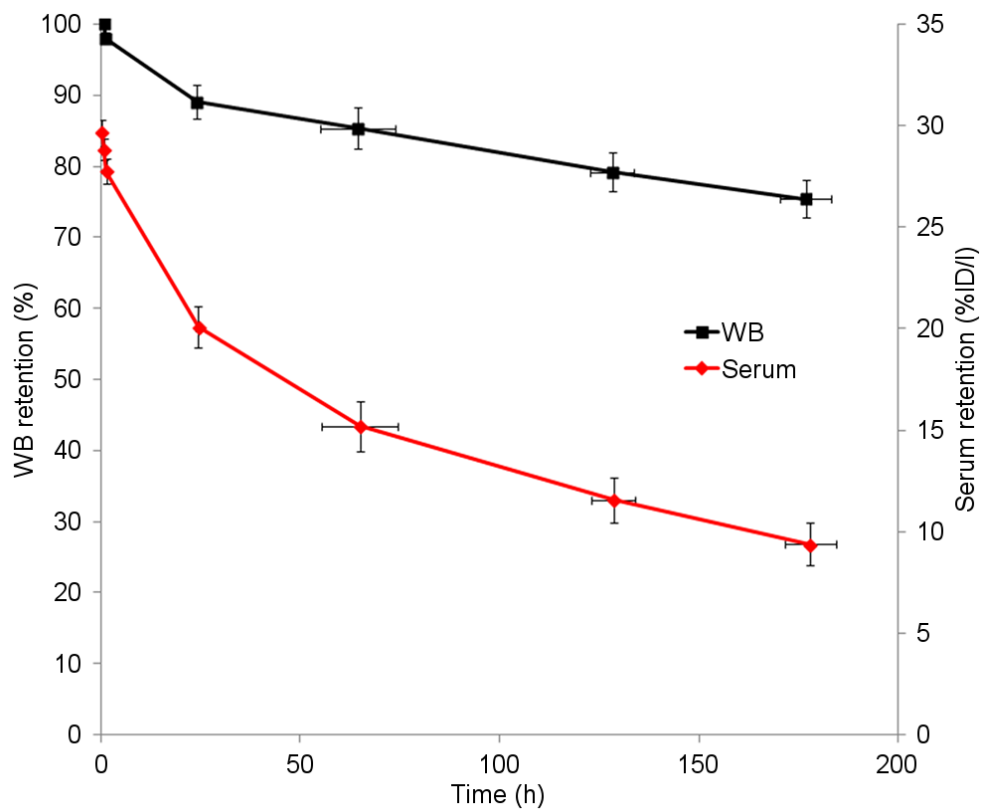


Figure 1. Summed whole body and serum biologic clearance data for ^{89}Zr -pertuzumab in six patients. Error bars indicate standard error of mean.

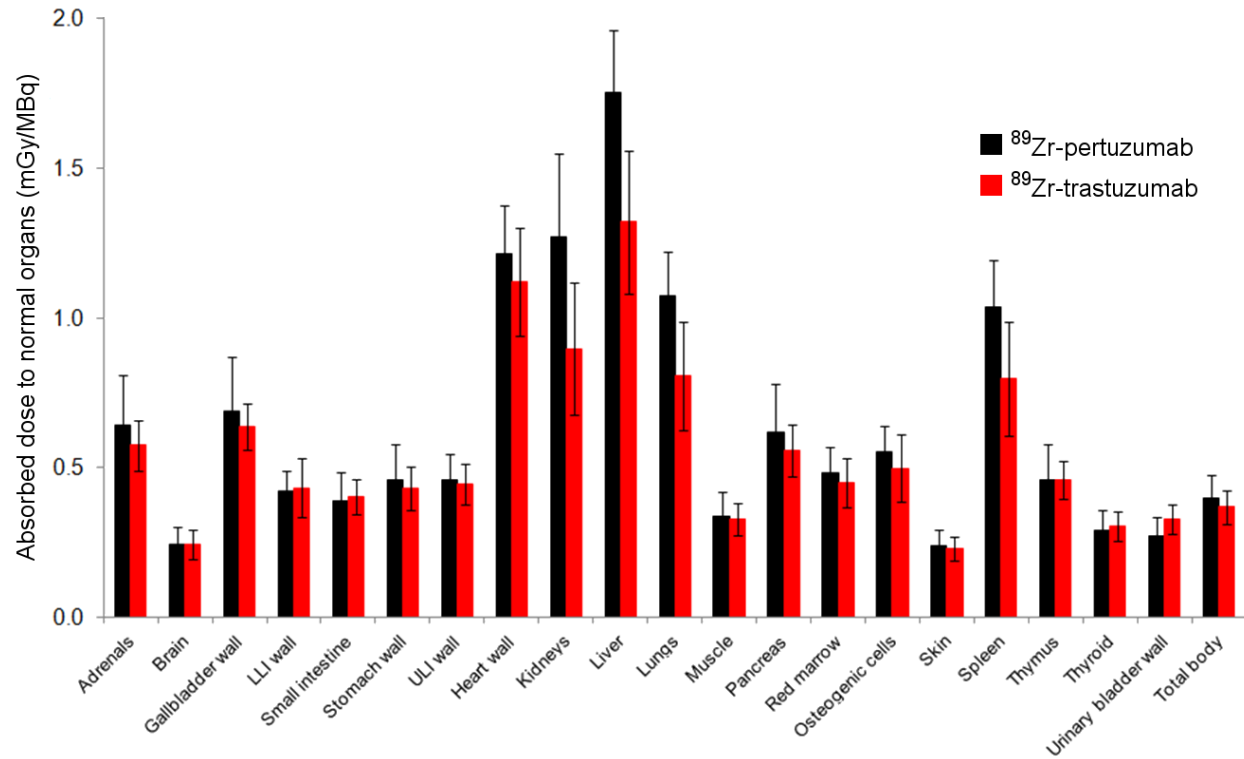


Figure 2. Comparative distributions of absorbed dose for ^{89}Zr -pertuzumab and ^{89}Zr -trastuzumab (16). Error bars denote standard deviations.

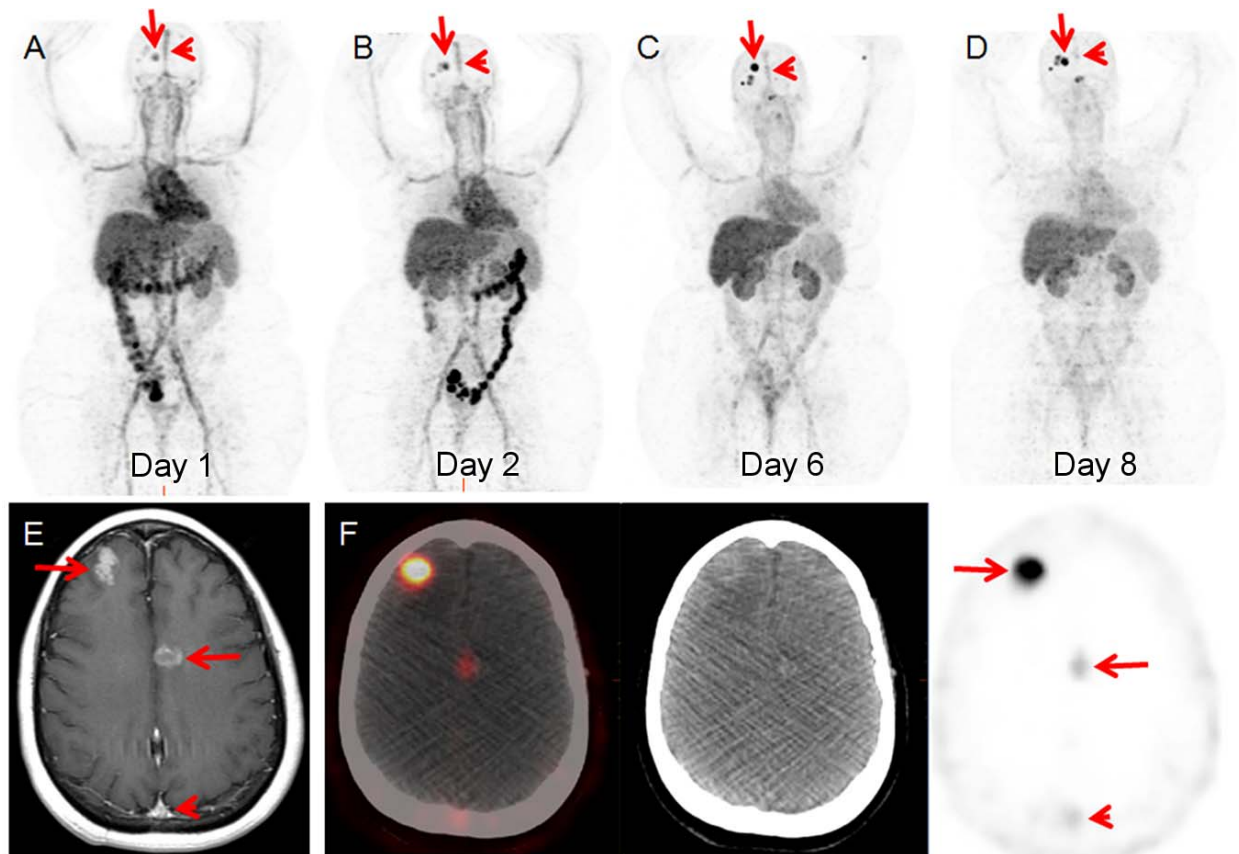


Figure 3. 46-year-old woman with both HER2-positive and HER2-negative primary breast malignancies and recently diagnosed brain metastases. Sequential maximum-intensity projection (MIP) images (A) 1 day, (B) 2 days, (C) 6 days, and (D) 8 days following administration of ^{89}Zr -pertuzumab. Blood pool and liver background clears on sequential images. Excreted bowel activity is seen on days 1 and 2. Bilateral kidney activity is visualized on all days. Increasing activity in foci overlying the skull is seen as time progresses (arrows). Decreasing activity is seen in the blood pool of the superior sagittal sinus (arrowheads). (E) Gadolinium-enhanced T1 weighed MR of the brain demonstrates enhancing brain metastases (arrows) and the superior sagittal sinus (arrowhead). (F) Axial fused PET/CT, CT, and PET images 8 days following ^{89}Zr -pertuzumab administration demonstrate avidity in the brain metastases (arrows) and minimal residual avidity in the superior sagittal sinus (arrowhead).

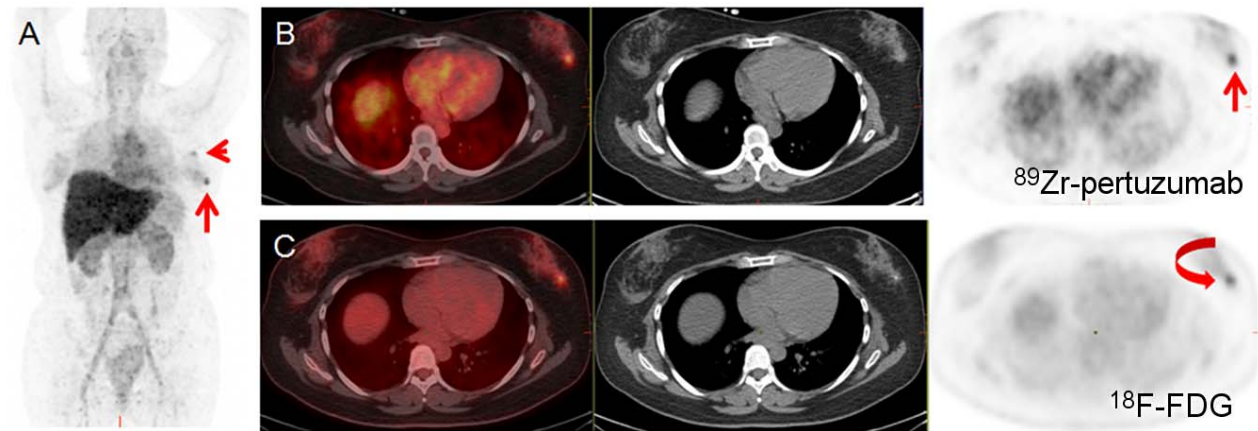


Figure 4. 58-year-old woman with HER2-positive breast cancer and current left breast and left axillary nodal disease. (A) MIP image 6 days following administration of ^{89}Zr -pertuzumab demonstrates the ^{89}Zr -pertuzumab-avid left breast (arrow) and left axillary nodal (arrowhead) disease. (B) Axial fused PET/CT, CT, and PET images obtained six days following ^{89}Zr -pertuzumab administration localize the ^{89}Zr -pertuzumab avidity in the left breast. (C) Axial fused PET/CT, CT, and PET images from a ^{18}F -FDG PET/CT scan three weeks prior demonstrates the corresponding ^{18}F -FDG-avid breast lesion.

Table 1: Characteristics of the Six Women with Invasive Ductal Breast Cancer

Patient	Age	Gender	Known Sites of Malignancy at Time of ⁸⁹Zr-pertuzumab Administration	Days Post-⁸⁹Zr-pertuzumab Administration of PET/CT Imaging	Sites of Demonstrably ⁸⁹Zr-pertuzumab-avid Disease
1	46	Female	Brain	1, 2, 6, 8	Brain
2	68	Female	Nodal	1, 2, 5, 7	Nodal
3	58	Female	Breast, nodal	1, 2, 6, 7	Breast, nodal
4	69	Female	Liver, bone, chest wall	1, 4, 5, 8	Chest wall
5	38	Female	Brain, lung, nodal, liver	1, 2, 5	Brain, lung, nodal
6	42	Female	Nodal	1, 4, 5, 7	None

Table 2: Summary Statistics for Whole Body and Serum Clearance

	Whole Body		Serum					
	$T_{1/2}$ eff	$T_{1/2}$ bio	A1 (%/L)	$T_{1/2}$ α eff	$T_{1/2}$ α bio	A2 (%/L)	$T_{1/2}$ β eff	$T_{1/2}$ β bio
Mean	67.7	521	12.6	12.1	15.5	17.3	59.3	201
SD	2.3	145	5.3	7.8	11.5	5.0	10.3	72
Median	66.9	456	13.0	9.7	11.2	16.3	55.5	176
Min	65.2	387	5.6	3.1	3.3	11.7	50.4	141
Max	70.8	730	18.8	22.1	30.8	23.3	78.4	322

Notes: Whole body clearance was mono-exponential. Serum clearance was bi-exponential conforming to the equation $A1\exp(-a1t) + A2\exp(-a2t)$. A1 and A2 are partition coefficients and the respective $T_{1/2}$ values correspond to $\ln(2)/a$. All half-times are in units of h.

Table 3: Absorbed Dose Estimates (mGy/MBq) for Normal Tissues

Target Organ	Mean	SD	Median	Min	Max
Adrenals	0.64	0.17	0.69	0.31	0.80
Brain	0.25	0.06	0.24	0.15	0.33
Breasts	0.33	0.08	0.35	0.18	0.41
Gallbladder Wall	0.69	0.18	0.72	0.34	0.83
Lower Large Intestinal Wall	0.42	0.06	0.40	0.36	0.52
Small Intestine	0.39	0.09	0.40	0.22	0.51
Stomach Wall	0.46	0.12	0.49	0.23	0.59
Upper Large Intestinal Wall	0.46	0.09	0.47	0.30	0.57
Heart Wall	1.22	0.16	1.24	0.98	1.37
Kidneys	1.27	0.28	1.18	0.98	1.64
Liver	1.75	0.21	1.73	1.54	2.13
Lungs	1.07	0.15	1.07	0.84	1.25
Muscle	0.34	0.08	0.35	0.19	0.44
Ovaries	0.38	0.10	0.38	0.20	0.50
Pancreas	0.62	0.16	0.66	0.30	0.77
Red Marrow	0.48	0.09	0.50	0.34	0.60
Osteogenic Cells	0.55	0.09	0.54	0.44	0.71
Skin	0.24	0.05	0.24	0.14	0.31
Spleen	1.04	0.16	1.01	0.87	1.26
Thymus	0.46	0.12	0.48	0.23	0.58
Thyroid	0.29	0.07	0.29	0.17	0.38
Urinary Bladder Wall	0.27	0.06	0.27	0.17	0.36
Uterus	0.37	0.10	0.37	0.19	0.49
Total Body	0.40	0.08	0.41	0.25	0.49
Effective Dose Equivalent (mSv/MBq)	0.72	0.09	0.73	0.56	0.83
Effective Dose (mSv/MBq)	0.54	0.07	0.56	0.41	0.62



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First-in-human HER2-targeted imaging using ^{89}Zr -pertuzumab PET/CT: Dosimetry and clinical application in patients with breast cancer

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